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III. THE INFLUENCE ON MILK YIELD OF HIGH AND LOW PROTEIN CONCENTRATES, EACH FED AT TWO LEVELS IN ADDITION TO DRIED GRASS AND GRASS SILAGE

BY D. REID AND W. HOLMES

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It has been stressed in recent years that if full use is to be made of the potential production from grassland, grass and grass products must form a greater proportion of the diet of dairy cows. Work on the problems involved has recently been reviewed by Cooper (1) who has shown the need for more research work on the subject. In experiments reported by Cooper, and described in detail recently by Danasoury (2), it was shown that a substantial proportion of the concentrate ration of cows could be replaced by a diet of hay, silage and fodder beet without loss in production. In the experiment reported here, which took place in 1953–54, the value of dried grass and silage in replacing concentrates was investigated.

EXPERIMENTAL

Cows. Eight Ayrshire cows from the Institute's herd were used. All had calved from 40 to 60 days before the experiment began. During the entire period the animals were housed in a byre provided with automatic water bowls, and were allowed out for short exercise periods in fine weather.

Experimental design. The eight cows were divided into two groups of four, so that each group was similar in stage of lactation and milk yield in the period preceding the experiment, and similar also in persistency as indicated in previous lactations. The cows were then arranged within each group according to a balanced Latin square. The square which was used for both groups was as follows:

	Periods			
Cows	1	2	3	4
1	A	B	C	D
2	B	D	A	C
3	C	A	D	B
4	D	C	B	A

This balanced square allows estimation of residual effects of treatments from period to period (Williams (3)). Each period lasted for 4 weeks, the change from one treatment to the next being made gradually during the first week.

Feeding. The four feeding treatments compared were as follows:

A 4 lb./gal. of high protein concentrate.
B 4 lb./gal. of low protein concentrate.
C 2 lb./gal. of high protein concentrate.
D 2 lb./gal. of low protein concentrate.

Details of the ingredients and nutrient composition of the two concentrate mixtures are given in Table 1. Both mixtures had a starch equivalent value of 63·2, whereas the digestible crude protein contents of the high- and low-protein mixtures were approximately 18·4 and 11·4% respectively.

During the first week of each period the cows were changed over gradually from one concentrate ration to another according to the sequence allocated to the particular animal. The actual amount of concentrate fed was adjusted according to milk yield.

Table 1. *Contents and nutrient composition of concentrate mixtures*

Contents	High-protein mixture	Low-protein mixture
	(parts by weight)	
Oats	3	3
Barley	2	3
Dried grass	2	2
Fish meal	¼	⅛
Beans	1	½
Decorticated earthnut cake	1	¼
Nutrient composition (from tables, Woodman(8))	%	%
Dry matter	87·3	86·9
Crude protein	18·8	13·9
Digestible crude protein	15·9	11·3
Digestible oil	2·3	2·4
Digestible carbohydrate	41·9	46·5
Digestible fibre	5·6	5·6
Starch equivalent	63·2	63·2
Crude protein as fed (by analysis)	21·8	14·1
Approximate digestible crude protein*	18·4	11·4

* Assuming the digestibility indicated by the tables.

This was done on the equalized feeding method described by Lucas(4), the amount of concentrate fed in any one period being based on the average decline in milk yield of each group over the preceding period. No alterations were made in the level of concentrate feeding during the remaining 3 weeks of each period. The concentrate ration was divided in two parts, fed before the morning and afternoon milking. In addition, two feeds of silage were given per day at 7.30 and 11.00 a.m., and two feeds of dried grass at 1.00 and 5.30 p.m. The amounts offered at every feed were weighed, the weights being adjusted during the introductory week of each period so that a small residue was left at each feed. This was taken to indicate that the cows were, in fact, being fed to appetite. The concentrates were eaten completely. Silage and dried grass residues were weighed after every feed on 3 days in each experimental week. The average crude protein contents of the dried grass and silage in each period are shown in Table 2.

On the days in which residues were collected, the silage and dried grass offered to the cows at each feed were sampled, and the dry-matter content of the samples determined. The dried samples from each feed were saved, and all dried grass and silage samples were bulked and subsampled after the residue-collecting period in each experimental week. The resulting subsamples were redried, ground and analysed for crude-protein content. Samples were also taken from the two concentrate mixtures at each feed over the three residue-collecting days. These were bulked, subsampled, and the subsamples analysed for dry-matter and crude-protein content.

From this programme of residue-collecting and feed sampling sufficient data were

obtained to allow the daily consumption by each animal of dry matter, grass dry matter and crude protein to be calculated, and, after making certain assumptions, the starch-equivalent and digestible crude-protein consumption were also estimated.

Milk yield and quality. The cows were milked twice daily, and milk yields recorded to the nearest 0·25 lb. at each milking. Milk samples for chemical analysis were taken for each cow from consecutive evening and morning milkings once in the third experimental week of periods 2–4. Evening and morning samples were mixed in proportion to yield, and analysed for fat and solids-not-fat. On account of the incomplete nature of the information so obtained it is not presented in this paper. However, the butterfat per-

Table 2. *Nutrient composition of grass feeds*

	C.P.	D.C.P.	S.E.
		(% of dry matter)	
Silage			
Period 1	9·5	5·4	50
Period 2	11·0	6·6	50
Period 3	11·3	6·9	50
Period 4	13·2	8·4	50
Average	11·2	6·8	50
'Afternoon' dried grass			
Period 1	11·8	6·5	56·4
Period 2	11·4	6·5	56·2
Period 3	17·0	12·5	59·6
Period 4	18·4	12·5	60·4
Average	14·6	9·5	58·2
'Evening' dried grass			
Period 1	17·0	12·5	59·5
Period 2	16·9	12·5	59·5
Period 3	18·1	12·5	60·2
Period 4	17·5	12·5	59·9
Average	17·4	12·5	59·8

centages determined have been used in the calculation of starch equivalent requirements in periods 2–4. For period 1, use was made of figures obtained on a routine test (by the Gerber method), made by the Scottish Milk Records Association during the third experimental week of that period.

Live weights. The cows were weighed on the Friday of each week, after morning milking and before the 11.00 a.m. silage feed.

Calculation of level of feeding. As noted above, starch-equivalent consumptions were calculated from the total dry-matter consumptions per day for each cow. In the absence of full analyses of the silage, a starch-equivalent value of 50% of the dry matter was assumed. This is in fairly close agreement with the values suggested by Dodsworth & Campbell (5) and Holmes (6). The starch-equivalent values for dried grass in each experimental week were calculated from the crude-protein content by the equation given by Watson (7), namely:

$$\text{Corrected S.E.} = 0{\cdot}6084x + 49{\cdot}222,$$

where x is the crude protein content in the dry matter. (In arriving at this equation Watson applied a correction of 0·29 lb. S.E. for each 1% of fibre in the grass dry matter.) The starch-equivalent values for the two concentrates were calculated on a dry-matter basis from feedingstuffs tables (8).

An estimate was also made of the starch equivalent required by each animal in each period from its average live weight (per period) milk yield and the butterfat content of the

milk according to the standards suggested by Woodman (8). The actual starch equivalent consumed by each animal was then expressed as a percentage of its calculated starch-equivalent requirement. This percentage has been taken as a measure of the level of feeding of starch equivalent. An example of the calculation is given in detail by Holmes, Waite, MacLusky & Watson (9) in a previous paper in this series.

Similar calculations were made for the consumption of digestible crude protein. For this it was assumed that for the low-grade dried grass of 11·5% crude protein fed in periods 1 and 2 the digestibility of the protein was 55%, and that for the high-grade dried grass fed in the evening throughout the experiment and in the afternoon in periods 3 and 4 the digestibility of the protein was 70%. For grass silage, digestible crude protein was calculated from the equation given by Watson (7):

$$\text{D.C.P.} = 0{\cdot}8179x - 2{\cdot}4154,$$

where x = the crude protein content of the dry matter.

RESULTS

Feed consumption

Total dry matter. The average daily consumption per cow of each feed in each treatment is included in Table 6. The daily consumption of total dry matter per cow for each treatment and the averages for each period are given in Table 3. Analysis of variance was first carried out on the data from second, third and fourth weeks of each period separately. By this means it was expected that any progressive change in consumption over the period would be detected. Finally an analysis of variance was carried out on the daily dry-matter consumptions per cow averaged over the 3 experimental weeks of each period.

For the individual weeks and for the entire period a highly significant difference in daily dry-matter consumption was shown to exist between cows fed concentrates at the rate of 4 lb./gal. milk produced (i.e. treatments A and B), and those fed 2 lb./gal. (treatments C and D). Since no consistent change was shown from week to week only the average figures for the 3 weeks are included in Table 3. On average, cows on treatments A and B consumed 2 lb. more dry-matter per day than those on treatments C and D.

Table 3. *Average feed consumption*

	Average consumption (lb./cow/day)			
Treatment	Total dry matter	'Grass' dry matter	S.E.	C.P.
A	31·4	18·5	19·5	5·6
B	31·7	19·2	19·7	4·8
C	29·4	23·0	17·4	4·8
D	29·9	23·4	17·8	4·5
Standard error of mean	±0·3	±0·4	±0·2	±0·05
A+B	31·6	18·9	19·6	5·2
C+D	29·6	23·2	17·6	4·7
Standard error of mean	±0·2	±0·3	±0·1	±0·04
Period				
1	31·8	20·7	19·5	4·8
2	31·8	21·5	19·4	4·8
3	31·2	21·4	18·8	5·3
4	27·7	20·4	16·9	4·8
Standard error of mean	±0·3	±0·4	±0·2	±0·05
C.V., %	3·0	5·3	2·6	2·9

No significant differences in daily dry-matter consumption ascribable to the protein level in the concentrates were detected in weeks 1 and 2 or over the entire period. In week 3, however, cows on treatment B had a total daily dry-matter consumption 1·4 lb. greater than cows on treatment A, but examination of the original data revealed that this difference was almost wholly due to an unusually high daily consumption of one cow in the third week of period 2. It may be assumed, therefore, that the two levels of protein concentrate fed had no differential influence on dry-matter consumption.

There was a highly significant difference in total dry-matter consumption between periods over all treatments. Average consumptions per day were identical in periods 1 and 2, but a decrease of 0·6 lb. occurred in period 3, and a large drop of 3·5 lb. in period 4. This is referred to below.

The coefficients of variation on the weekly analyses were all 3–4%, and over the 3-week period 3·0%.

Grass dry matter. The average daily consumption of grass dry matter for the four treatments over all periods is shown in Table 3, together with period averages. There was a highly significant difference between the grass dry-matter consumption of cows receiving 4 lb. concentrate/gal. and those receiving 2 lb./gal. The latter cows consumed, on average, 4·3 lb. more grass dry matter per day than the former. The protein content of the concentrate mixture did not significantly affect grass dry-matter consumption.

In comparison with total dry-matter consumption, grass dry-matter consumption showed no significant differences from period to period when averaged over all treatments. The decline in total intake in periods 3 and 4 noted above must, therefore, have been caused by decreased concentrate consumption.

Starch equivalent. The total starch-equivalent consumption per day per cow was calculated as already outlined, and treatment and period averages are included in Table 3. Cows fed 4 lb. concentrate/gal. milk consumed 2·0 lb. more starch equivalent per day than those fed only 2 lb./gal.

The starch-equivalent consumption per day for each period averaged over all treatments showed a slight but more-or-less steady decrease from periods 1 to 3, followed by a sharp drop in period 4.

Crude protein. The total crude-protein consumption per cow per day was calculated from the weekly analyses of feed samples. There were highly significant differences between cows receiving 4 lb. concentrate/gal. milk and those receiving only 2 lb. Averages given in Table 3 indicate that cows on treatments A and B (4 lb. concentrate/gal.) consumed 0·5 lb. more crude protein than those on treatments C and D (2 lb./gal.). The difference in crude-protein consumption between cows on the high-protein concentrate mixture and those on the low was also highly significant. The degree of this difference varied with the actual amount of concentrate fed. At the 4 lb./gal. level, the high-protein mixture provided 0·8 lb. more crude protein per day than the low-protein mixture; while at the 2 lb./gal. level the difference was only 0·3 lb. in favour of the high-protein mixture.

As a result of the increasing content of protein in the silage with periods and the use of high-protein dried grass for the afternoon feed in periods 3 and 4, there was no decline in protein intake with periods, and indeed the intake in period 3 exceeded that of the other periods.

The influence of dry-matter content of the silage on the amount consumed. It was shown by

analysis of variance that within periods, significant day-to-day variations occurred in the dry-matter percentage of the silage fed, and that the sampling error was low. The effect of these variations on the amount of silage consumed per day was, therefore, investigated.

Regression analyses of the amount of silage dry matter eaten per day on the dry-matter percentage of the silage were calculated separately in each period for animals receiving 4 lb. concentrates per day and for those receiving 2 lb. per day. The regression coefficients obtained, and their levels of significance, were as follows:

Period	4 lb./gal.	2 lb./gal.
1	0·38*	0·71*
2	0·15 (NS)	0·16 (NS)
3	0·01 (NS)	0·08 (NS)
4	0·23**	0·34**

* Significant at 5% level.
** Significant at 1% level.

The smaller effect on silage consumption of variations in silage dry-matter percentage with cows receiving 4 lb. concentrates/gal. is probably due to silage forming a smaller proportion of the total ration than with cows receiving 2 lb. concentrates/gal., the other feeds masking the effect.

No satisfactory explanation can be found for the non-significant regressions obtained in periods 2 and 3. It was thought that the cows might have consumed a smaller proportion of silage to other feeds in these two periods, but investigation proved that this was not so. Some other factor such as palatability may have exerted a greater influence on silage consumption than dry-matter percentage in these two periods.

Milk yields

The average milk yields per cow for the 3-week periods for each treatment are given in Table 4. Examination of the data indicated that there were no differences in the trend of results from week to week. Analysis by the method of Williams (3) showed no significant residual effect of treatments from period to period.

On the average, cows fed concentrates at the rate of 4 lb./gal. yielded 1·2 lb. more milk per day than cows fed 2 lb./gal., and this difference was statistically highly significant. It will be noted from Table 4 that there was a tendency for cows receiving the high protein concentrate to yield rather more milk than those on the low-protein mixture, but this difference was small and only significant (at the 5% level) when concentrates were fed at the rate of 4 lb./gal.

Milk yields declined steadily from period to period.

The coefficient of variability for milk yields calculated from the average milk yield over the three experimental weeks of each period for each treatment was 3·7%. This is surprisingly low in view of the rather limited choice of animals available for the experiment.

Live weights

Analysis of variance of live weights for each cow, averaged over the three experimental weeks of each period, showed no significant differences between treatments or between periods. The results included in Table 4 indicate, however, a slightly higher average live weight for animals receiving 2 lb. concentrates/gal. of milk produced compared with those

fed 4 lb./gal. The actual difference amounted to only 7 lb. The results also suggest slightly higher average live weights for cows on the low-protein concentrate mixture as compared with those on the high-protein mixture. At the 4 lb./gal. feeding level this difference was 7 lb., but at the 2 lb./gal. level it was only 2 lb.

Table 4. *Animal production and intensity of feeding*

Treatment	Milk yield (lb./cow/day)	Live weight (lb.)	Level of feeding, % Woodman standards		Dry matter eaten (lb./100 lb. live weight)
			S.E.	D.C.P.	
A	34·2	1091	126	149	2·87
B	33·0	1098	130	129	2·89
C	32·7	1100	112	122	2·68
D	32·2	1102	118	115	2·70
Standard error of mean	±0·4	±5	±1·6	±2·5	±0·27
A + B	33·6	1094	128	139	2·88
C + D	32·4	1101	115	118	2·70
Standard error of mean	±0·3	±4	±1·1	±1·8	±0·19
Period					
1	39·2	1089	116	108	2·92
2	35·3	1095	119	114	2·90
3	31·2	1098	126	142	2·85
4	26·5	1110	125	151	2·50
Standard error of mean	±0·4	±5	±1·6	±2·5	±0·27
c.v., %	3·7	1·4	3·7	5·4	2·8

Regression analyses of live weight on time tended to confirm the above trends. The coefficients obtained are shown in Table 5. The regressions calculated by pooling the data over all periods indicated that no significant live-weight gains or losses resulted from the

Table 5. *Regression coefficients of live weight on weeks*

Treatment	(lb./cow/week) Period				Over all periods
	1	2	3	4	
A	9·4*	−1·1	−6·0	4·5*	1·7
B	8·0	10·6*	3·1	6·2†	7·0**
C	−8·0	4·9	3·9	−3·6	−0·7
D	7·5	2·3	9·8	−0·1	4·9†

** Highly significant (at 1% level).
* Significant (at 5% level).
† Almost significant (at 5% level).

high-protein concentrate mixture at either 4 or 2 lb./gal. (i.e. treatments A and C), but the low-protein mixture fed at 4 and 2 lb./gal. gave live-weight increases of 7 and 4·9 lb./week respectively. The first of these coefficients was significant at the 1% level, and the second almost significant at 5%.

The results of these regression analyses must be treated with caution in view of the variation from period to period, i.e. between animal pairs, and also within periods, i.e. within animal pairs. However, it should be stated that comparison of the regressions by analysis of variance showed significant differences between or within animal pairs on only one treatment, a significant difference (at the 5% level) between pairs on treatment A.

Level of feeding

The level of feeding of starch equivalent was calculated as described, and the averages for each treatment and period are shown in Table 4. Cows fed 4 lb. concentrate/gal. received a higher level of feeding compared with those receiving 2 lb./gal., the difference being significant at the 1% level. Animals on the low-protein mixture also were slightly more overfed than those on the high protein, but this was only significant (at the 5% level) in the 2 lb./gal. feeding level.

The period changes, given in Table 4, show an increase in the level of feeding from the first to the third period, with the fourth period almost equal to the third. Appetite apparently exceeded nutrient requirements as the lactation progressed. An alternative explanation is that the value of the roughages was overestimated.

Since dried grass of higher protein content was used in periods 3 and 4 there was a significant difference in level of feeding of digestible crude protein between periods 1 and 2, and 3 and 4. There was a linear increase in the level of feeding of digestible crude protein from ration D to ration A, but even the former supplied 115% of the requirement of digestible crude protein, while the latter supplied 149%.

The average dry-matter consumption for each treatment and each period expressed as a percentage of live weight is also shown in Table 4. Animals on rations A and B consumed a significantly greater amount of dry matter in relation to their live weight than animals on rations C and D. The different levels of protein in the concentrate had no significant effect on these values. The ratio of dry matter eaten to live weight declined fairly steadily from period to period, whereas average live weight increased slightly.

DISCUSSION

With the present feeds, increasing the rate of concentrate feeding from 2 to 4 lb./gal. increased the total dry-matter consumption by 2 lb./day, and the average milk yield by only 1·2 lb. For every pound increase in concentrate fed there was a decrease of 0·68 lb. in grass dry matter eaten. The protein content of the concentrate had no effect on intake, and only a slight, though positive, effect on milk yield.

Before discussing the practical implications of these results, it should be stressed that the bulk feeds offered to the cows in this experiment were dried grass of good quality (17% C.P.) and silage of medium quality. The cows showed a marked preference for the former. Under average farm conditions a more likely combination of bulk feeds would be hay and silage, and it would be of interest to discover whether cows on the low level of concentrate feeding would be capable of maintaining their milk yields on these lower quality bulk feeds.

The difficulties associated with the determination of animal live weights must also be taken into consideration. As noted previously, there were no significant differences in average live weight between treatments or between periods, and only with treatment B was a significant 'live weight on time' regression coefficient obtained, although that for treatment D approached significance. This tendency for the low-protein concentrate mixture to produce live-weight increase at the 4 and 2 lb./gal. feeding levels is in remarkable agreement with the data for level of feeding of starch equivalent presented in Table 4. The amount by which the starch equivalent fed exceeded the requirements according to

the Woodman standards was greater on the low- than on the high-protein concentrate ration. This was due to the slight reduction in milk yield which occurred on the low-protein concentrate ration.

The higher levels of protein feeding did not apparently encourage live-weight gain to the same extent as the lower levels. The failure of the high-protein ration to give much increase in milk yield is not surprising since even on ration D the requirements for protein were amply met. In view of these results it is probable that a concentrate ration consisting mainly of cereals, provided it was palatable, would be adequate when fed in conjunction with grass feeds of high quality.

The changeover type of design used in this experiment undoubtedly caused the difficulties in detecting live-weight changes. A period of 4 weeks with only weekly weighings was apparently too short for live-weight changes of statistical significance to develop, since the treatments applied did not differ widely. However, the results obtained show that the design used was sufficiently sensitive to detect small changes in milk yield and feed consumption. Where several feeding treatments were under comparison, designs of the changeover type might, therefore, be used for an investigation of their effects on milk yield, but if the results from such experiments suggested that live-weight changes of economic importance would develop under certain treatments, it might be desirable to conduct further investigations with these treatments on a continuous feeding system.

Although differences in milk yield caused by feeding 4 lb. instead of 2 lb. concentrate/gal. were statistically significant, it is doubtful whether they are of any practical value. If 4 lb./gal. is taken as an average rate of concentrate feeding, the results of this experiment have indicated that halving it and feeding more bulk foods caused only a slight reduction in milk yield, but lessened feed costs considerably. This is well illustrated by the costing presented in Table 6. In this table the daily cost of feeding each of the various rations is

Table 6. *The relation between feed cost and the value of the milk produced*

	Ration							
	A		B		C		D	
Yield of milk (lb./day)	34·2		33·0		32·7		32·2	
Value of milk (at 4·9 pence/lb.)	168		162		160		158	
	Daily ration, weight and cost							
	lb.	pence	lb.	pence	lb.	pence	lb.	pence
Dried grass	14·2	28·4	14·2	28·4	17·7	35·4	18·2	36·4
Silage	32·8	10·9	35·4	11·8	39·9	13·3	39·4	13·1
Concentrates	14·7	51·5	14·2	42·6	7·2	25·2	7·4	22·2
		90·8		82·8		73·9		71·7
Total								
Margin over food cost (pence)	77		79		86		86	

Dried grass	£18·66 per ton.
Silage	£3·10 per ton.
Low-protein concentrate	£28·00 per ton.
High-protein concentrate	£32·66 per ton.

compared with the value of the average amount of milk produced daily on each ration. Where concentrates were fed at 4 lb./gal. the average margin of milk value over feed costs was 78 pence. At the 2 lb./gal. level, however, the margin was 86 pence.

The range of protein level of the two concentrate mixtures was not great, and significant differences in milk yield occurred only at the 4 lb./gal. feeding level. The costings in

Table 6 show, however, that feeding the low-protein concentrate (treatments B and D) left almost the same margin of milk value over feed cost as the high-protein mixture (treatments A and C).

The conclusion to be drawn from these results, therefore, is that, provided the diet was made up with high-quality dried grass and silage, feeding either the high- or the low-protein concentrate mixture at the rate of 2 lb./gal. milk gave the greatest profit margin per cow. The profit on a 'per acre' basis would probably be greatest under this feeding system also, in view of the higher yields and lower production costs of the grass feeds in comparison with cereal crops.

SUMMARY

1. In a winter-feeding experiment with eight Ayrshire cows the effects of feeding a high- and a low-protein concentrate at two rates (4 and 2 lb./gal.), in addition to dried grass and silage, were studied.

2. With these cows and the feeds used increasing the rate of concentrate feeding from 2 to 4 lb./gal. increased the total dry-matter consumption by 2 lb./day and the average milk yield by 1·2 lb./day.

3. For every 1 lb. increase in concentrate fed there was a decrease of 0·68 lb. grass dry matter eaten.

4. The level of protein in the concentrates had no effect on total dry-matter intake, and only a slight, though positive, effect on milk yield.

5. It is concluded that in this work where the diet was made up with high-quality dried grass and silage, feeding either a high- or a low-protein concentrate mixture at the rate of 2 lb./gal. milk gave the greatest profit margin per cow.

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622. INFECTION AND MASTITIS IN A DAIRY HERD 1945–53

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(With 2 Figures)

INTRODUCTION

The sources of variation in the incidence of udder infection and mastitis in dairy cattle can be broadly classified as due either to individual differences between cows or to environment. The former covers such factors as the anti-bacterial and stimulatory properties of milk, acquired resistance through previous infection, teat patency, and other general physiological factors which may affect susceptibility (i.e. age, stage of lactation, pregnancy, level of production). The environmental factors are either uncontrolled, such as air temperature, rainfall, season, or factors which, at least in part, are under the control of the milker. These are hygiene, the type and virulence of the micro-organism, the methods and type of milking, and possibly nutrition. A review of the known effects of these sources of variation has been made recently by Oliver (1).

It is possible to investigate the importance of these various factors in two ways: first, by analysing records of mastitis incidence collected over a number of years in one or more herds; and secondly, by carrying out experiments designed specifically to measure the effects of one or two particular factors. The former method is useful for measuring the effects of certain physiological factors (i.e. age, stage of lactation, level of production) and those environmental factors which are uncontrolled, but it is normally not suitable for measuring the effect of the more complex environmental factors which need the experimental approach.

The data presented below were collected from a large herd in which detailed records of infection and mastitis were made over the period 1945–53. Details are given of the management, methods of mastitis diagnosis and analysis of the data. The results show the amount of infection and mastitis that occurred, and it is analysed not only on an annual basis but for monthly periods within each year and also according to age. From these data it is not possible to arrive at definite conclusions on the effect of age, season and other factors on the incidence of mastitis because of the effects of interactions. They are, however, presented below as an introduction to the later papers in which the more detailed analyses will be given.

THE DESCRIPTION OF THE HERD

The dairy herd

The investigation of the herd began in October 1945 and continued for 8 years until September 1953. At first the herd was composed of Shorthorn and Guernsey cattle in the proportion of two-thirds and one-third respectively, but in 1950 a number of British Friesians were purchased and the composition of the herd gradually changed until, at the

end of the investigation, there were 60% Shorthorns, 34% British Friesians and only 6% Guernseys. Heifers calving after 1 October 1950 were removed from the herd to other premises so that from this date onwards no first-lactation animals entered the herd.

The experimental work which was carried out in the herd was mainly nutritional, and it is unlikely that it affected the present investigation. In the main the management of the animals was on lines typical of commercial practice. The cattle were attested and free from contagious abortion. Further details of the breeding and management of the herd have been given by Mackintosh (2), Foot & Ridler (3), Braude & Walker (4) and Bailey (5).

Milking routine

The herd was milked in one large shed containing a double row of standings, together holding fifty-seven cattle. Machine milking was introduced in October 1945, and the herd was gradually changed over to machine milking until, in July 1947, only ten cows were hand milked, and these had completed their lactations by February 1948. A bucket plant was used at a vacuum of 14–15 in. of mercury with pulsators adjusted to give about 40 cyc./min.; moulded liners were used in the teat cups.

The following milking routine was prescribed. On entering the shed the animal's hindquarters were superficially cleaned using a jet of cold water from a hose. Each udder was washed with hot water immediately before it was milked. Until 1951 the foremilk was examined on a strip-cup on two occasions each week, but since October of that year a strip-cup was used before each milking. Disinfectants were not used, and there was no segregation of infected animals. Machine stripping was practised.

The number of animals in milk

The number of animals in milk each month of each year is shown in Table 1, and the mean number of animals in milk each year shows how herd numbers increased. The totals for calendar months illustrates that there were most animals in milk in May and least in December, but the monthly variations were small.

Table 1. *The number of animals in milk each month of each year, October* 1945 *to September* 1953

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Mean
1945–46	44	40	42	47	24	31	26	26	30	48	44	45	37
1946–47	44	47	47	42	45	54	57	58	55	52	45	55	50
1947–48	56	54	49	49	47	49	53	54	52	43	41	45	50
1948–49	50	54	55	57	54	58	65	67	64	63	59	62	59
1949–50	67	68	66	73	63	66	74	75	65	52	54	63	65
1950–51	69	75	72	77	80	83	89	89	88	85	84	82	81
1951–52	73	72	73	78	90	101	104	108	108	103	100	93	92
1952–53	90	86	71	82	87	95	97	97	96	90	91	88	89
Total	493	496	475	505	490	537	565	574	558	536	518	533	

The total number of animals in milk at each stage of lactation and according to month of calving is shown in Table 2. Of the 530 animals calving in this period 444 had lactations of 8 months or longer. The mean length of lactation was 9·1 months and varied from 1 to 19 months.

The number of animals calving each month

Table 3 illustrates the total number of first, second, third and fourth and over calvings each month, while Table 4 shows the number of calvings each month of each year.

During the period under review the herd policy was to mate cattle for autumn calving, and this was most successful with the younger animals. Calvings were highest in January and August due to the calving down of older cows and heifers respectively. May, June and July were the months when fewest animals calved.

Table 2. *The numbers of animals in milk at each stage of lactation according to month of calving, October* 1945 *to September* 1953

Month of calving	1st month	2nd month	3rd month	4th month	5th month	6th month	7th month	8th month	9th month	10th month	11th month	12th month	13th month	14th month
Jan.	63	63	63	60	59	58	57	51	41	27	13	3	1	—
Feb.	54	54	53	53	53	53	51	51	39	25	14	7	2	—
Mar.	53	53	53	53	53	52	49	47	35	20	9	1	1	—
Apr.	45	45	45	45	45	42	40	37	34	14	3	3	—	—
May	23	23	23	23	22	22	21	17	14	8	6	4	3	2
June	10	10	10	10	9	9	9	8	5	1	1	—	—	—
July	22	22	22	21	21	20	16	15	13	9	8	4	1	1
Aug.	60	59	59	57	56	55	51	46	39	34	16	9	7	3
Sept.	50	50	50	49	49	47	47	45	34	21	10	6	5	2
Oct.	42	42	42	41	41	41	40	38	32	19	6	1	—	—
Nov.	59	59	57	57	56	55	52	47	35	24	11	5	1	—
Dec.	49	49	49	49	49	49	45	42	29	28	13	7	5	1
Total	530	529	526	518	513	503	478	444	340	230	112	50	26	9

Table 3. *The number of animals calving each month*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1st calvers	10	6	8	2	3	—	7	26	15	16	16	15	124
2nd calvers	16	14	14	12	3	2	4	15	19	11	20	12	142
3rd calvers.	15	13	11	5	7	3	8	8	6	8	10	7	101
4th and over	22	21	20	26	10	5	3	11	10	7	13	15	162
Total	63	54	53	45	23	10	22	60	50	42	59	49	530

Table 4. *The number of animals calving each month from October* 1945 *to September* 1953

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1945	—	—	—	—	—	—	—	—	—	3	6	4	13
1946	9	2	5	—	2	1	2	6	5	8	6	3	49
1947	6	7	8	7	2	3	—	6	14	6	8	3	70
1948	3	9	8	5	2	—	—	7	9	6	8	9	66
1949	4	9	8	6	7	—	8	8	8	11	12	10	91
1950	6	5	2	9	4	2	2	21	7	1	7	6	72
1951	7	7	6	5	3	2	5	7	2	2	3	8	57
1952	15	8	10	8	2	1	3	5	5	5	9	6	77
1953	13	7	6	5	1	1	2	—	—	—	—	—	35
Total	63	54	53	45	23	10	22	60	50	42	59	49	530

BACTERIOLOGICAL METHODS

Milk samples

Foremilk samples were taken aseptically as previously described (Crossman, Dodd, Lee & Neave(6); Neave, Dodd & Henriques(7)). In the first 2 years milk samples were taken every 2–4 months, and subsequently at intervals of 1–2 months or more frequently.

One or more additional samples were taken before a cow became dry, when dry, before calving and again after calving. Normally three or more samples were obtained when a new infection was suspected.

Examination of milk samples

About 0·05 ml. of each sample was plated on aesculin ox-blood agar. Colonies that developed after 2 days at 37° C. were identified as described in earlier publications (6, 7). The Whiteside test was the main supplementary test used to detect abnormality. It was used on all samples taken during lactation until April 1952; thenceforth it was applied to the first few milk samples from new infections only.

Infection

A quarter was assumed to be infected if: (*a*) bacteria were recovered in large numbers from two or more milk samples and the supplementary tests were positive (about 90% of new infections were in this class); (*b*) there were clear clinical signs of disease but only a few micro-organisms were recovered (about 0·5% of cases) before treatment began; (*c*) micro-organisms were recovered once in large numbers or several times in small numbers, but generally the indirect or supplementary tests were positive. These cases (about 10%) could be interpreted as temporary or transitory infections (Table 5).

Table 5. *Quarters with temporary or transitory 'infections'*

Case	Supplementary tests (highest reading)		Bacterium	Number of colonies on blood agar plates (successive samples in a period of 5 weeks or less)
	Whiteside	Conductivity difference (6)		
1	3	21	*Staphylococcus aureus*	60, 11, 50, 19, 0, 0, 0, 0
2	1	5	*Staph. aureus*	40, 60, 18, 7, 4, 2, 2, 0, etc.
3	3	2·5	*Staph. aureus*	500, 0, 0, 0, 0
4	4	N.T.	*Streptococcus dysgalactiae*	∞, 0, 0, 0, 0
5	4	5	*Str. dysgalactiae*	100, 0, 0, 0, 0
6	4	N.T.	*Corynebacterium pyogenes*	120, 0, 0, 0, 0

N.T. = no test.

Mastitis

A cow was regarded as having mastitis if a quarter showed marked inflammation or induration, or if the milk contained obvious clots. Symptoms of disease found in the last week of lactation, however, have not been taken into consideration unless (*a*) they occurred in an infected quarter only, or (*b*) were much more severe in the infected than in the other quarters.

Treatment

Normally, until February 1946, acute cases of mastitis only received treatment, using aqueous solutions of penicillin; subsequently, all cases of mastitis (infection with clinical signs of disease) were treated. When one course of treatment failed to effect a cure, further courses were generally given, usually until the micro-organism could no longer be recovered.

All acute cases of mastitis in dry animals were treated with antibiotics. In addition, between July 1949 and June 1951, two quarters of each cow drying-off were infused with penicillin, but this is unlikely to have affected the present analysis materially.

Bacteriological cure

From 1945 to March 1948 usually three to four samples of milk were taken during the first month after treatment of infected quarters or when a spontaneous recovery was suspected. Subsequently, five samples were taken at weekly intervals. The absence of the infecting micro-organism from these samples was taken to indicate that it had been eliminated from the quarter.

Other observations

After overnight refrigeration, all samples were observed for abnormal appearance and flakes, clots and discoloration recorded. Obvious clinical signs observed in the cowshed were noted, as were injuries, sores, chaps and erosions of the teat apex. Sores and erosions were noted whenever milk samples were taken.

METHODS OF ANALYSIS

The bacteriological and clinical history of each quarter of each animal was plotted on a chart horizontally (example, Fig. 1). Vertical lines were drawn to represent monthly calendar divisions, but in order to keep the chart size to a practical minimum the scale of time was kept small so that it was not possible, nor was it considered to be necessary, to be more accurate than ± 3 days in plotting the data. This method of plotting made it possible immediately to see the month and year of calving, while the listing of animals of similar age and month of calving was facilitated.

From these charts lists of animals were made on analysis paper (Fig. 2) according to month of calving, so that the history of each quarter of each animal could be recorded as a sequence of events in terms of successive months from calving. With the aid of a transparent scale, graduated in intervals representing 1 month, the stage of lactation at which new infections took place were read off the history chart and recorded on the analysis paper. Infections were classified into 'first infections' and 're-infections'. For cow data a 'first infection' was the first that the cow contracted in its lifetime, and all subsequent infections were classified as re-infections in a previously infected cow irrespective of whether the originally infected quarter was involved. With the quarter data a first infection was the first to occur in a specific quarter and each succeeding infection in that quarter was regarded as a re-infection of a previously infected quarter. Thus for each successive month of each lactation for each animal it was possible to analyse the number of lactating quarters; the number infected or free from infection at the beginning of the month; the number of quarters that had been previously infected, together with the number of times the particular quarter had been infected; the number of quarters which became infected; the type of micro-organism causing infection; the number of quarters affected with mastitis and the number infused with antibiotics. Herd examinations prior to 1945 were infrequent and the previous infection history of a few cows was not known but these were insufficiently numerous to affect the results materially.

A specimen chart is shown in figs. 1 and 2 for ten third-lactation cows calving in the month of February for the years 1945–53. The analysis of these data is shown in Table 8.

The method of analysis is illustrated for the cow Valerie (Fig. 1, last row). From previous charts it was found that Valerie had been infected in each quarter before commencing her third lactation. The micro-organisms concerned were *Streptococcus uberis* in

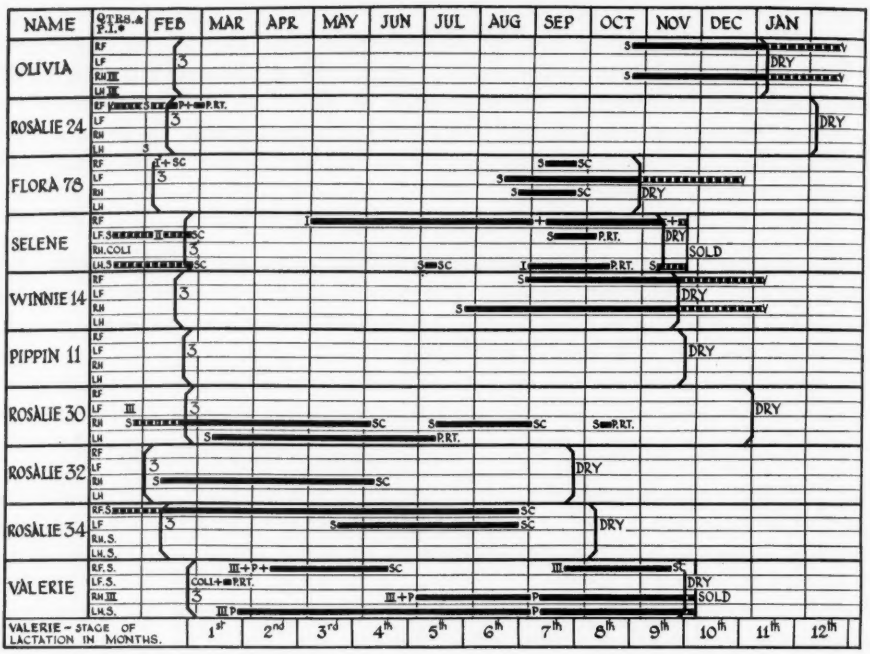

Fig. 1. First stage of the analysis. Chronological plotting of the records from each animal. S = *Staph. aureus.* I = *Str. agalactiae.* II = *Str. dysgalactiae.* III = *Str. uberis.* C.py = *C. pyogenes.* Coli = *Bacterium coli*, type I, II or intermediate. (RF) alone = RF with mastitis in a quarter already infected. + = clinical symptoms. P = antibiotic treatment. RT = response to treatment. SC = spontaneous recovery.

* QTRS. & P.I. = quarter concerned and previous infection.

the case of the right hind (RH) quarter which had been infected on three separate occasions, and *Staphylococcus aureus* in the other three quarters. None of these quarters was infected when the lactation began. In the first month of lactation the RF quarter became clinically affected with *Str. uberis*, the LH infected with *Str. uberis* and the LF quarter clinically affected with *Bacterium coli* type I. These infections were treated with an antibiotic but only the coliform infection was eliminated. Thus at the beginning of the second month of lactation the cow had two quarters which were uninfected, the RH had been previously infected three times, and each of the remaining quarters had been infected twice. This position remained unaltered until the beginning of the fourth month of lactation, and in this month the RH quarter developed a clinical infection with *Str. uberis*, which was treated but not cured. At the same time a spontaneous recovery took place in the RF quarter. At the beginning of the fifth month of lactation there were still two uninfected quarters, the RH had been infected on four occasions and the remaining quarters infected twice. No new infection occurred until the seventh month of lactation when the RF quarter became infected with *Str. uberis;* the RH and LH quarters were treated with penicillin but the infections were not eliminated. At the beginning of the eighth month of lactation only one quarter was not infected, and the RF quarter had now been infected on three previous occasions. There were no further changes for the remainder of the lactation apart from the spontaneous recovery of the RF quarter in the ninth month of lactation, and the cow was sold when dry.

Month of Lactation		1					2					3					4					5					6					7					8					9					10					11					12				
		Quarters					Quarters					Quarters					Quarters					Quarters					Quarters					Quarters					Quarters					Quarters					Quarters					Quarters					Quarters				
Year	Name of cow	Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected		Uninfected	Becoming infected	Micro-organisms	Times previously infected	
					LF	RF				LH	RH				LH	RH				LH	RH				LH	RH				LH	RH				LH	RH				LH	RH				LH	RH				LH	RH				LH	RH
1948	Olivia	4					4					4					4					4					4					4					4					4					2					2									
1948	Rosalie 24	3	RF				4					4					4					4					4					4					4					4					4					4					1
1949	Flora 78	4	RF	I			4					4					4					4					4					4	RM S / LF S				2	RF	S			3															
1949	Selene	2					4					4	RF	I			3					3	LH	S			3					3	RM / LF	I / S			1					3	LH	S													
1949	Winnie 14	4					4					4					4					4					4	RH	S			3	RF	S			2					2														
1951	Pippin 11	4					4					4					4					4					4					4					4					4														
1951	Rosalie 30	3	LH	S			2					2					2					3	RH	S			3					3					4	RH	S			4					4									
1952	Rosalie 32	4	RH	S			3					3					3					3					4					4					4																		
1952	Valerie	4	RF LF	III Calf III			2					2					2	RH	III			2					2					2	RF	III			1					1														
1953	Rosalie 34	3					3					3					3	LF	S			2					2					2					4																		
Total uninfected		35					34					34					33					33					34					33					30					25					10					6					4				
Total quarters in milk		40					40					40					40					40					40					40					40					32					12					8					4				
New Infections	Previously uninfected	3										1					1										1					3					2					1														
	Previously infected	3															1					2										3					2					2														
Total previously infected qtrs.		10					19					19					20					21					21					21					24					20					8					5					2				
Number of times previously infected quarters had been infected	Once	14					14					14					15					16					14					15					18					12					6					4									
	Twice	1					4					4					4					4					6					6					3					3					1					1									
	Three times	1					1					1					1																					3					4					1									
	Four times																					1					1					1					1					1														
Number of quarters clinical		4															1																																									

Fig. 2. Second stage of analysis. Tabulation of data from Fig. 1. Key to symbols on Fig. 1.

Table 6. *The number of cows in milk, their mean lactation age, the number infected with different organisms and the number of clinical cases each 2 months from October 1945 to September 1953*

Type of infection	Oct.–Nov.		Dec.–Jan.		Feb.–Mar.		Apr.–May		June–July		Aug.–Sept.		Total of all cows in milk	
	Cows infected	Cows clinical	Cows infected	Cows clinical	Cows infected	Cows clinical	Cows infected	Cows clinical	Cows infected	Cows clinical	Cows infected	Cows clinical	Cows infected	Cows clinical
1945–46														
Agalactiae	5 (10·6)	2	9 (17·6)	4	3 (6·4)	1	5 (10·9)	2	7 (15·6)	4	3 (6·8)	1	13 (22·4)	11
Green	1 (2·1)	1	4 (7·8)	1	3 (6·4)	1	3 (6·5)	1	4 (8·9)	3	3 (6·8)	—	5 (8·6)	3
Staph.	2 (4·3)	1	7 (13·7)	2	4 (8·5)	—	5 (10·9)	1	3 (6·7)	1	3 (6·8)	1	9 (15·5)	4
Others	1 (2·1)	1	—	—	—	—	—	—	—	—	—	—	1 (1·7)	1
Total	7 (14·9)	5 (10·6)	14 (27·5)	5 (9·8)	9 (19·1)	2 (4·3)	11 (23·9)	3 (6·5)	11 (24·4)	6 (13·3)	8 (18·2)	2 (4·5)	22 (37·9)	16 (27·6)
Cows in milk	47		51		47		46		45		44		58	
Mean age lact.	2·8		2·9		2·7		2·7		2·7		2·5		2·7	
1946–47														
Agalactiae	2 (4·0)	—	3 (6·1)	2	4 (8·2)	2	7 (11·7)	4	5 (8·9)	3	2 (3·6)	—	9 (13·4)	8
Green	4 (8·0)	1	4 (8·2)	—	5 (10·2)	1	5 (8·3)	4	5 (8·9)	2	5 (8·9)	1	11 (16·4)	7
Staph.	3 (6·0)	—	4 (8·2)	2	4 (8·2)	—	6 (10·0)	3	5 (8·9)	1	4 (7·1)	1	10 (14·9)	4
Others	1 (2·0)	—	1 (2·0)	1	1 (2·0)	—	—	—	—	—	—	—	2 (3·0)	1
Total	7 (14·0)	1 (2)	9 (18·4)	5 (10·2)	10 (20·4)	3 (6·1)	14 (23·0)	7 (11·6)	14 (25·0)	5 (8·9)	9 (16·1)	2 (3·6)	26 (38·8)	12 (17·9)
Cows in milk	50		49		49		60		56		56		67	
Mean age lact.	2·7		2·6		2·7		2·8		2·9		2·9		2·8	
1947–48														
Agalactiae	4 (6·8)	2	3 (5·9)	—	2 (3·7)	1	2 (3·6)	2	1 (2)	—	1 (2·3)	1	4 (6·0)	4
Green	3 (5·1)	1	3 (5·9)	—	6 (11·1)	2	4 (7·1)	1	1 (2)	—	7 (16·3)	2	14 (20·9)	6
Staph.	10 (16·9)	2	12 (23·5)	2	11 (20·3)	2	13 (23·2)	2	13 (26·0)	—	16 (37·2)	6	32 (47·8)	12
Others	1 (1·7)	—	—	—	—	—	—	—	—	—	1 (2·3)	—	4 (6·0)	—
Total	13 (22·0)	3 (5·1)	15 (29·4)	2 (3·9)	15 (27·8)	4 (7·4)	17 (30·4)	3 (5·4)	13 (26·0)	—	19 (44·2)	9 (20·9)	38 (56·7)	17 (25·4)
Cows in milk	59		51		54		56		50		43		67	
Mean age lact.	2·5		2·6		2·6		2·8		2·7		3·2		2·7	
1948–49														
Agalactiae	2 (3·4)	1	—	—	2 (3·1)	1	9 (12·5)	3	6 (8·9)	1	12 (18·8)	3	13 (13·5)	7
Green	6 (10·3)	2	9 (14·3)	2	12 (18·5)	—	12 (16·7)	3	10 (14·9)	3	15 (23·4)	7	26 (27·1)	11
Staph.	21 (36·2)	8	21 (33·3)	5	24 (36·9)	2	33 (45·8)	8	24 (35·8)	3	42 (65·6)	9	56 (58·3)	23
Others	2 (3·4)	1	1 (1·6)	1	—	—	1 (1·4)	—	—	—	1 (1·6)	1	7 (7·3)	2
Total	24 (41·4)	8 (13·8)	25 (39·7)	8 (12·7)	28 (43·1)	3 (4·6)	35 (48·6)	12 (16·7)	31 (46·3)	6 (8·9)	42 (65·6)	14 (21·9)	64 (66·7)	30 (31·2)
Cows in milk	58		63		65		72		67		64		96	
Mean age lact.	3·0		2·5		2·5		2·8		2·7		2·7		2·7	

Table 6 (*cont.*)

Table 6 (*cont.*)

Type of infection	Oct.–Nov. Cows infected	Oct.–Nov. Cows clinical	Dec.–Jan. Cows infected	Dec.–Jan. Cows clinical	Feb.–Mar. Cows infected	Feb.–Mar. Cows clinical	Apr.–May Cows infected	Apr.–May Cows clinical	June–July Cows infected	June–July Cows clinical	Aug.–Sept. Cows infected	Aug.–Sept. Cows clinical	Total of all cows in milk Cows infected	Total of all cows in milk Cows clinical
1949–50														
Agalactiae	7 (10)	1	2 (2·7)	1	2 (3·1)	1	2 (2·6)	1	2 (3·5)	—	1 (1·4)	1	7 (6·6)	3
Green	9 (12·9)	—	12 (16·2)	4	7 (10·8)	—	10 (13·0)	5	7 (12·3)	2	8 (11·1)	3	18 (17·0)	8
Staph.	30 (42·9)	6	33 (44·6)	8	30 (46·2)	6	29 (37·7)	5	24 (42·1)	5	24 (33·3)	6	58 (54·7)	23
Others	—	—	—	—	1 (1·5)	—	1 (1·3)	1	2 (3·5)	—	2 (2·8)	2	2 (1·9)	2
Total	35 (50·0)	6 (8·6)	36 (48·6)	11 (14·9)	28 (43·1)	6 (9·2)	34 (44·2)	8 (10·4)	27 (47·4)	7 (12·3)	25 (34·7)	7 (9·7)	65 (61·3)	19 (17·9)
Cows in milk	70		74		65		77		57		72		106	
Mean age lact.	2·6		2·2		2·1		2·1		2·5		2·6		2·3	
1950–51														
Agalactiae	1 (1·4)	—	—	—	—	—	1 (1·3)	—	1 (1·5)	—	1 (1·4)	—	1 (1·1)	—
Green	9 (12·5)	2	6 (8·7)	—	13 (17·6)	3	14 (18·2)	2	14 (21·2)	2	17 (24·3)	5	24 (25·3)	7
Staph.	29 (40·3)	7	33 (47·8)	5	31 (41·9)	1	33 (42·9)	1	29 (43·9)	7	33 (47·1)	6	53 (55·8)	21
Others	1 (1·4)	—	2 (2·9)	—	2 (2·7)	1	—	—	—	—	—	—	2 (2·1)	1
Total	34 (47·2)	7 (9·7)	35 (50·7)	5 (7·2)	35 (47·3)	42 (54·5)	4 (5·4)	3 (3·9)	37 (56·1)	8 (12·2)	43 (61·4)	9 (12·9)	66 (69·5)	25 (26·3)
Cows in milk	72		69		74		77		66		70		95	
Mean age lact.	2·4		2·6		2·7		2·9		3·1		3·1		2·9	
1951–52														
Agalactiae	1 (1·8)	—	1 (1·7)	—	—	—	1 (1·3)	—	1 (1·3)	—	—	—	1 (1·1)	—
Green	19 (33·9)	7	17 (28·8)	5	21 (29·6)	6	21 (26·3)	6	22 (29·3)	5	19 (27·5)	5	43 (45·7)	24
Staph.	25 (44·6)	7	18 (30·5)	5	30 (42·3)	11	39 (48·8)	12	33 (44·0)	7	26 (37·7)	2	62 (65·9)	28
Others	—	—	3 (5·1)	1	3 (4·2)	2	3 (3·8)	1	2 (2·7)	—	—	—	8 (8·5)	4
Total	31 (55·4)	12 (21·4)	30 (50·8)	9 (15·3)	42 (59·2)	17 (23·9)	48 (60·0)	16 (20·0)	43 (57·3)	11 (14·7)	34 (49·3)	7 (10·1)	74 (78·7)	49 (52·1)
Cows in milk	56		59		71		80		75		69		94	
Mean age lact.	3·3		3·4		3·3		3·4		3·5		3·7		3·7	
1952–53														
Agalactiae	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Green	9 (14·5)	1	13 (28·9)	4	16 (27·1)	2	17 (24·6)	6	13 (19·4)	4	16 (20·0)	6	30 (32·6)	17
Staph.	24 (38·7)	2	22 (48·9)	6	26 (44·1)	4	37 (53·6)	10	32 (47·8)	9	39 (48·8)	8	58 (63·0)	37
Others	—	—	—	—	—	—	1 (1·4)	1	1 (1·5)	1	1 (1·3)	—	1 (1·1)	1
Total	28 (45·2)	3 (4·8)	26 (57·8)	7 (15·6)	33 (55·9)	6 (10·2)	42 (60·9)	12 (17·4)	35 (52·2)	9 (13·4)	44 (55·0)	12 (15·0)	69 (75·0)	45 (48·9)
Cows in milk	62		45		59		69		67		80		92	
Mean age lact.	3·7		4·1		3·7		3·8		3·8		3·8		3·9	
Summary														
Agalactiae	22	6	18	7	13	6	27	12	23	8	20	6	—	—
Green	60	15	68	16	83	15	86	28	76	21	90	29	—	—
Staph.	144	33	150	35	160	26	195	42	163	33	187	39	—	—
Others	6	2	7	3	7	3	6	3	5	1	5	3	—	—
Total	179	45	190	52	200	45	243	64	211	52	224	62	—	—
Cows in milk	474	—	461	—	484	—	537	—	483	—	598	—	—	—

The figures in parentheses are the percentage of total cows in milk. Agalactiae = *Str. agalactiae*; green = *Str. dysgalactiae*, *Str. uberis* and group D streptococci; Staph. = *Staphylococcus aureus*; others = *Corynebacterium pyogenes*, *Escherichia coli*, *Aerobacter aerogenes*, *Coliform bacteria* type I and II and intermediate.

The results obtained from each quarter of each cow were analysed in the same way for each of the 530 lactations studied. For each month of calving, the data required were added up as shown on the analysis sheet, and these totals were then summarized for each lactation group. These data are presented in Table 6. Similar data for cows were derived from the analysis sheets while the overall incidence of infection and mastitis according to type of micro-organism (Table 7) was taken directly from the chart.

Table 7. *The number of infected lactations according to age and type of micro-organism and the number of lactations in which new infections took place*

Age in lactations	No. of lactations	*Streptococcus agalactiae*	'Green'* *Streptococcus*	*Staphylococcus aureus*	Other micro-organisms	No. of infected lactations	No. of lactations with new infections
1st	124	11 (8·9)	13 (10·5)	45 (36·3)	5 (4·0)	55 (44·4)	52 (41·9)
2nd	142	5 (3·5)	29 (20·4)	68 (47·9)	11 (7·8)	80 (56·3)	61 (43·0)
3rd	101	4 (4·0)	34 (33·7)	51 (50·5)	2 (2·0)	68 (67·3)	54 (53·5)
4th and over	163	22 (13·5)	63 (38·7)	109 (66·9)	6 (3·7)	131 (80·3)	111 (68·1)
Totals	530	42 (7·9)	139 (26·2)	273 (51·5)	24 (4·5)	334 (63·0)	278 (52·5)

* *Str. dysgalactiae, Str. uberis* and group D streptococci.

Figures in parentheses are the numbers expressed as a percentage of the number of lactations in the group.

PRELIMINARY ANALYSIS

The total incidence of infection in cows

The level and type of infection for the various age groups in the herd is shown in Table 7. A total of fifty-five (44·4%) heifers were infected. Of these, fifty-two (41·9%) acquired new infections during lactation and three were infected before calving. The number of infected animals in each group increased with each lactation after the first. This may be partly due to herd policy, since Dodd & Foot (8) have shown that in this herd there was a tendency to cull the slow milkers at an early age, and these have been shown to be more resistant to infection (Dodd & Neave (9)). Thus the increasing number of 'infected lactations' with advancing age may be due to a change in herd population.

The annual incidence of infection and mastitis

Data are presented in Table 6 to show in each 2 months of the period the number of cows in milk, their mean lactation age, the number infected with different micro-organisms and the number of infections which gave rise to clinical symptoms.

The mean lactation age of the animals showed little variation until 1949–50, when an increased intake of heifers reduced the average age for the herd (see also Table 4). From this date herd replacements comprised second calving and older cows which resulted in a rise in herd age to 3·9 lactations by 1952–53.

There were 22·4% of the cattle infected with *Streptococcus agalactiae* in 1945–46, but the practice of treating clinical quarters with antibiotics gradually reduced the incidence of this micro-organism so that there were no cows remaining infected after July 1952. Staphylococcus infections increased from 15% in 1945–46 to about 55% in the years 1948–51. This increase was preceded by the introduction of machine milking during 1946. The increased incidence of infection during 1951–53 accompanied the advance in age of the herd.

The total incidence of infection and mastitis in quarters

The summarized data for first infections and re-infections of quarters of cows in milk are contained in Table 8, together with the number of quarters free from infection and the number of previously infected quarters. The data are shown for each age group of

Table 8. *New infections in quarters in each month of lactation for each lactation stage*

Months of lactation		1st calvers	2nd calvers	3rd calvers	4th calvers and over	Totals
1	New infections					
	Total quarters in milk	495	568	403	652	2118
	In quarters not infected previously	22	9	8	19	58
	In previously infected quarters	—	2	9	29	40
	No. of previously infected quarters	9	115	166	434	724
2	Total quarters in milk	495	564	403	652	2114
	In quarters not infected previously	12	13	6	2	33
	In previously infected quarters	4	2	7	34	47
	No. of previously infected quarters	31	123	174	453	781
3	Total quarters in milk	495	556	399	652	2102
	In quarters not infected previously	8	14	8	5	35
	In previously infected quarters	—	10	5	23	38
	No. of previously infected quarters	43	133	179	455	810
4	Total quarters in milk	491	540	391	648	2070
	In quarters not infected previously	4	13	5	4	26
	In previously infected quarters	1	5	7	31	44
	No. of previously infected quarters	49	142	186	456	833
5	Total quarters in milk	487	536	383	644	2050
	In quarters not infected previously	8	4	3	5	20
	In previously infected quarters	2	4	13	22	41
	No. of previously infected quarters	53	155	189	460	857
6	Total quarters in milk	487	524	371	628	2010
	In quarters not infected previously	4	13	4	2	23
	In previously infected quarters	1	9	5	20	35
	No. of previously infected quarters	61	152	185	451	849
7	Total quarters in milk	483	488	343	596	1910
	In quarters not infected previously	4	4	3	3	14
	In previously infected quarters	—	3	5	14	22
	No. of previously infected quarters	65	157	173	428	823
8	Total quarters in milk	470	440	307	564	1781
	In quarters not infected previously	3	4	1	7	15
	In previously infected quarters	—	5	6	17	28
	No. of previously infected quarters	69	148	161	403	781
9	Total quarters in milk	418	328	223	420	1389
	In quarters not infected previously	1	5	6	3	15
	In previously infected quarters	1	6	4	23	34
	No. of previously infected quarters	67	115	111	306	599
10	Total quarters in milk	327	216	115	260	918
	In quarters not infected previously	6	1	1	1	9
	In previously infected quarters	1	2	1	8	12
	No. of previously infected quarters	55	101	63	184	408
11	Total quarters in milk	160	104	63	120	447
	In quarters not infected previously	2	—	—	—	2
	In previously infected quarters	—	1	1	3	5
	No. of previously infected quarters	31	52	36	86	205
12 and over*	Total quarters in milk	—	—	—	—	—
	In quarters not infected previously	2	1	—	2	5
	In previously infected quarters	1	—	1	6	8
	No. of previously infected quarters	—	—	—	—	—
	Total for complete lactations. New infections					
New infections	In non-infected quarters	76	81	45	53	255
	In previously infected quarters	11	49	64	230	354
	No. of previously infected quarters	85	196	211	487	979

* Data for 12th and succeeding months.

animals for each month they are in milk. The number of quarters is less than would be expected from an inspection of the number of cows in milk (Table 2) because blind quarters have been excluded from the analysis. During the period there were 255 quarters infected for the first time and 354 re-infections in previously infected quarters. The total number of previously infected quarters for all lactations, 979, represents the sum of the total number of previously infected quarters at calving, 724, and the total number of first infections, 255, in the period.

The number of previously infected quarters at the beginning of the first month of all lactations was 724, while the number of first infections in that month was 58. Thus the number of previously infected quarters at the beginning of the second month of all lactations was $724+58=782$, but one of these quarters had to be excluded, making the actual total 781. Quarters had to be excluded when they became dry or blind or were otherwise not available. The data in Table 8 have been analysed, and the results will be published elsewhere (Oliver, Dodd, Neave & Bailey(10)).

SUMMARY

A description has been given of a dairy herd, its breed composition, history and management over a period of 8 years. A close study of infection and mastitis was made in the herd, and the bacteriological methods used have been outlined. The data collected have been analysed and the methods of analysis have been described. There were 530 complete lactations for which full records were available and, because of the frequency of milk sampling and the care with which the bacteriological and other tests were made, these data are considered to be suitable for a precise study of the effect on the incidence of infection and mastitis of stage of lactation, season of the year, age and previous infections. These factors will be the subject of a later report.

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623. VARIATIONS IN THE INCIDENCE OF UDDER INFECTION AND MASTITIS WITH STAGE IN LACTATION, AGE AND SEASON OF THE YEAR

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(With 5 Figures)

INTRODUCTION

In a previous publication (1) we described the history of a dairy herd, its management and the methods by which the udder health records were collected and analysed. This report comprises analyses of the data to study the relationship of stage of lactation, age and season of the year with udder infection and mastitis.

THERAPEUTIC TREATMENT OF INFECTED QUARTERS

Treatment was confined to infected quarters that showed clinical signs of disease, except that as previously stated (1), during 1949–51, two quarters of each dry cow were infused with penicillin.

GENERAL METHOD OF ANALYSIS

The data were analysed for first infection rate, re-infection rate, total new infection rate and the total incidence of infection and mastitis for both cows and quarters. Complete udder health histories were available for all lactations, so that it was possible to differentiate between 'first infections' and 're-infections' in the history of a cow or quarter. 'Total new infections' are all infections that *started* in a given period of time and total infection is the sum of these infections with those *already present* when the period began. Mastitis incidence is the proportion of total infection associated with clinical signs of disease. Infection caused by coagulase-negative micrococci and non-haemolytic diphtheroids has been excluded.

STAGE IN LACTATION

There were not sufficient data to analyse stage in lactation effects for the different age groups or for season of calving. This analysis, therefore, was made from the pooled data of all cows. The number of cows and quarters examined, the number becoming infected, the total number infected and the number with mastitis were tabulated (Table 1) for each month after calving. To illustrate the trends present in the infection rate and mastitis incidence, these data were expressed as percentages and plotted in Fig. 1. The results obtained after the ninth month of lactation were variable because of the small numbers of cows and quarters available. A separate analysis has been made from the data of the last 4 months of lactation.

RESULTS

(i) *Cows.* The number of cows becoming infected for the first time (Fig. 1, Table 1), was 11% in the first month of lactation and fell gradually to 1·8% in the ninth month. Similar trends were apparent for re-infections and total new infections. The incidence

Table 1. *Udder infection and mastitis in cows and quarters according to stage in lactation*

Months from calving	Classification: Free from infection*	Classification: Previously infected	Classification: No. in milk	First infection: Infected	First infection: % of no. free from infection	Re-infection: Re-infected	Re-infection: % of previously infected	Total new infection: Newly infected	Total new infection: % of no. in milk	Total infection: No. infected (old and new infection)	Total infection: % of no. in milk	Mastitis: Mastitis cases	Mastitis: % of total infection
						Cows							
1	246	284	530	27	11·0	42	14·8	69	13·0	200	37·7	61	30·5
2	219	310	529	13	5·9	56	18·1	69	13·0	185	35·0	29	15·7
3	206	320	526	14	6·8	47	14·7	61	11·6	202	38·4	20	9·9
4	191	327	518	10	5·2	49	15·0	59	11·4	200	38·6	33	16·5
5	179	334	513	11	6·1	40	12·0	51	9·9	206	40·2	25	12·1
6	170	333	503	8	4·7	39	11·7	47	9·3	202	40·2	26	12·9
7	156	322	478	6	3·9	24	7·5	30	6·3	191	40·0	24	12·6
8	139	307	446	3	2·2	35	11·4	38	8·5	182	40·8	26	14·3
9	110	237	347	2	1·8	32	13·5	34	9·8	139	40·1	17	12·2
		χ^2 value		20·56 — $P<0·01$		19·88 — $P<0·01$		20·42 — $P<0·01$		Not significant —		45·23 — $P<0·01$	
						Quarters							
1	1394	724	2118	58	4·2	40	5·5	98	4·6	347	16·4	83	23·9
2	1333	781	2114	33	2·5	47	6·0	80	3·8	300	14·2	28	9·3
3	1292	810	2102	35	2·7	38	4·7	73	3·5	320	15·2	22	6·9
4	1237	833	2070	26	2·1	44	5·3	70	3·4	335	16·2	39	11·6
5	1193	857	2050	20	1·7	41	4·8	61	3·0	344	16·8	32	9·3
6	1161	849	2010	23	2·0	35	4·1	58	2·9	345	17·2	32	9·3
7	1087	823	1910	14	1·3	22	2·7	36	1·9	311	16·3	32	10·3
8	1000	781	1781	15	1·5	28	3·6	43	2·4	301	16·9	29	9·6
9	790	599	1389	15	1·9	34	5·7	49	3·5	235	16·9	20	8·5
		χ^2 value		33·91 — $P<0·01$		16·49 — $P<0·05$		28·54 — $P<0·01$		Not significant —		68·98 — $P<0·01$	
						Cows. The last four months of lactation							
4	161	317	478	11	6·8	27	8·5	38	7·9	183	37·6	23	12·4
3	150	328	478	2	1·3	38	11·6	40	8·4	193	39·6	20	10·4
2	148	330	478	5	3·4	27	8·2	32	6·7	192	39·4	23	12·0
1	143	335	478	7	4·9	41	12·2	48	10·1	198	40·7	33	16·7
		χ^2 value		None of these values was significant at the 5% level									

* Free from infection: *cows*—that had never been infected; *quarters*—that were not infected at the beginning of the period examined.

of total infection was fairly constant apart from a decline in the second month. This is probably due to the treatment and cure of many mastitis cases in the first month of lactation, and the frequent spontaneous recovery from infection of cows that calved with persisting dry-period infections.

In the first month of lactation 30% of all infected cows showed mastitis symptoms, whereas the values for the other months were usually between 10 and 15%.

Multiple χ^2 values were calculated to test this relationship between stage of lactation and the various measures of infection rate. Apart from the values for total infection all of these trends were highly significant ($P < 0{\cdot}01$).

The last 4 months of lactation. The method of analysing the data for lactation trends given above enables a reliable estimate to be made for the first 7 months of lactation since the number of animals included did not decline appreciably until after this time. In

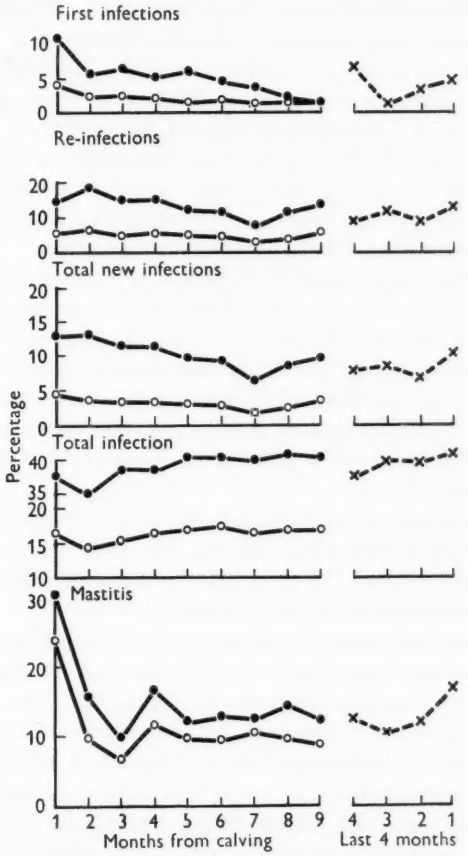

Fig. 1. Udder infections and mastitis in cows and quarters according to age in lactation. ●, cows; ○, quarters; ×, cows last 4 months.

order to discover whether towards the end of lactation there was a departure from the general trend of decreasing infection rate and mastitis from the beginning of lactation, the data were analysed in another way.

The results were tabulated for the last 4 months of lactation only, and animals that were in milk for 6 months or less were excluded so that the data would not be biased by early lactation effects. These data are presented in Table 1, and the infection and mastitis rates were expressed as percentages and plotted in Fig. 1. The slight rises in incidence in the final month of lactation are not statistically significant.

(ii) *Quarters.* The analysis of the data on a quarter basis is also given in Table 1 and Fig. 1. The trends shown are similar to those observed for cows.

Discussion

Although it has been suggested that a cow's resistance to infection and mastitis may vary with stage in lactation there is little agreement in the published data on this subject. Stylianopoulos (see Munch-Petersen (2)), Ward & Castle (3), Murphy (4) and Withers (5) found a high incidence in early lactation, while a herds survey by Watts (6) showed the incidence to be greatest in the first 3 and last 3 months of lactation. Kästli (7) found the incidence to increase after the sixth month of lactation, while Minett, Stableforth & Edwards (8) and Ineson & Cunningham (9) did not demonstrate a significant 'stage-of-lactation' effect. These greatly differing results are due chiefly to lack of uniform methods for the diagnosis of infection and mastitis, and to errors in the interpretation of the data which arise when dry-period infections cannot be distinguished from lactation infections because animals were tested at insufficiently frequent intervals.

The data examined in this paper are, we believe, free from serious bias in that sampling was relatively frequent, infections occurring before the animals calved have been definitely excluded and a distinction made between first and re-infections.

The data for both cows and quarters show that the rate of new infection and mastitis declines with advance in lactation. With the exception of re-infections in previously infected quarters the decline is greatest in early lactation, particularly from the first to the second month in lactation. It is possible that there is also a rise in infection in the last month of lactation.

The data for re-infections of previously infected cows and quarters show peaks in the second month of lactation after which the rate falls with considerable variations. The general pattern is undoubtedly one of decline from the beginning of lactation which supports the data obtained from the first infections.

The mean rate of first infections was 5·8 and 2·3 % per month for cows and quarters respectively, and the corresponding figures for the re-infection rate was 13·1 and 4·7 %. The higher mean rates for re-infections are probably mainly due to the fact that they apply to the inherently more susceptible animals (ie. those that have already been infected).

It has been suggested by Steyn (10) and Rose (11) that a high incidence of udder infection and mastitis in early lactation might be due to the rapid physiological changes which take place in the mammary tissue *post partum*, resulting in reduced udder resistance. On the other hand, the increase may be due to the stimulatory effect of colostrum on bacterial growth (Auclair (12)). This stage of lactation effect appears quite definitely to be of more importance than that of duration to exposure which would produce the reverse result to that which was found.

THE EFFECT OF LACTATION AGE ON THE INCIDENCE OF UDDER INFECTION AND MASTITIS

Two methods of analysing the data for the relationship between age and the incidence of udder infection and mastitis have been carried out. In the first, the number and percentage of cows and quarters that acquired infection and mastitis have been calculated for each lactation age group, and the results obtained are shown in Table 2 and Fig. 2. Strictly this method does not measure the effect of age, but relates the infection in comparatively

unselected heifers with that of selected older age groups. Since there is evidence of a tendency to cull more resistant animals (Dodd & Neave(13); Dodd & Foot(14)) these results may be subjected to bias. This disadvantage was overcome by the second method of analysis in which the infection was calculated for each of the first four lactations of

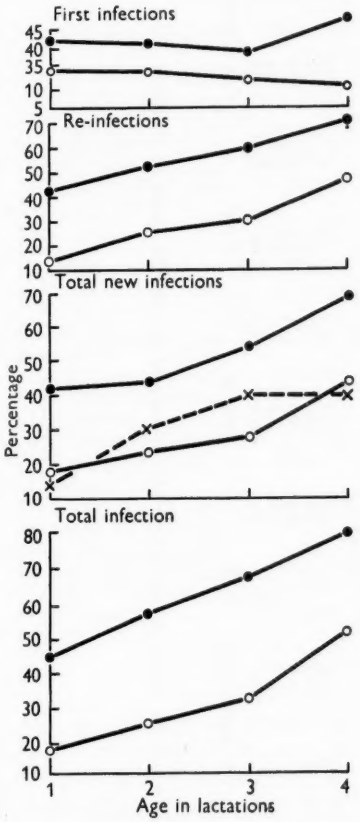

Fig. 2. Udder infection and mastitis in cows and quarters according to age in lactation. ●, cows; ○, quarters; ×, forty-two cows.

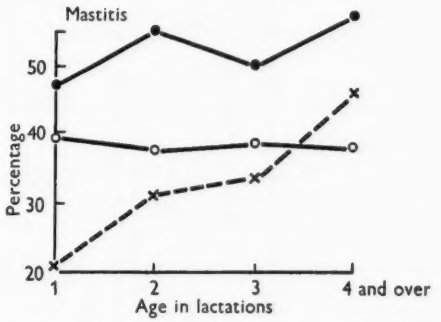

Fig. 3. Mastitis in cows and quarters according to age. ●, cows infected; ○, quarters infected; ×, cows in milk.

forty-two animals. This method overcomes the bias introduced by culling. Nevertheless, the analysis is based on the records collected from a selected sample (i.e. animals having at least four lactations) which may not be respresentative of the population. The results of this second analysis are given in Table 3 and Fig. 2.

It will be seen from the results of the first analysis (Table 2 and Fig. 2) that there was a significant rise in re-infections, total new infections and total infection with increase in lactation age which was true for both cows and quarters. The apparent declines in the first infection rate for quarters and for the first three lactations in cows were not significant. The proportional mastitis incidence in infected quarters was similar for the four age groups studied (Fig. 3). When, however, the mastitis incidence was expressed as a

Table 2. *Udder infection and mastitis in cows and quarters according to age in lactations*

Lactations	Classification: Free from infection*	Classification: Previously infected	Classification: No. in milk	First infection: Infected	First infection: % of no. free from infection	Re-infection: Re-infected	Re-infection: % of previously infected	Total new* infections: No. infected (old and new infections)	Total new* infections: % of no. in milk	Total infections†: Newly infected	Total infections†: % of no. in milk	Mastitis: Mastitis cases	Mastitis: % of total infection	Mastitis: % of cows in milk
Cows														
1	115	9	124	48	41·7	4	44·4	52	41·9	55	44·3	26	47·3	21·0
2	79	63	142	28	39·2	33	52·4	61	43·0	80	56·3	44	55·0	31·0
3	29	72	101	11	37·9	43	59·7	54	53·5	68	67·3	34	50·0	33·7
4	22	141	163	11	50·0	100	70·9	111	68·1	131	80·4	75	57·3	46·0
Total	245	285	530	98	40·0	180	63·2	278	52·5	334	63·0	179	53·6	33·8
		χ^2 value		Not significant		8·97 — $P<0·05$		29·18 — $P<0·01$		43·11 — $P<0·01$		Not significant		24·94 $P<0·01$
Quarters														
1	486	85	495	76	15·6	11	12·9	87	17·6	84	17·0	33	39·3	
2	532	196	568	81	15·2	49	25·0	130	22·9	141	24·8	53	37·6	
3	355	211	403	45	12·7	64	30·3	109	27·0	129	32·0	50	38·8	
4	495	487	652	53	10·7	230	47·2	283	43·4	337	51·7	128	38·0	
Total	1868	979	2118	255	13·7	354	36·2	609	28·8	691	32·6	264	38·2	
		χ^2 value		Not significant		59·37 — $P<0·01$		108·62 — $P<0·01$		178·77 — $P<0·01$		Not significant		

* Free from infection as for Table 1.
† Total infection shows the number of cows and quarters found to be infected at some time during the lactation.

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percentage of the total cows in milk the expected significant increases with age were found ($P < 0{\cdot}01$).

The second analysis (Table 3 and Fig. 2) shows highly significant increases in the total new infections with increase in age. Thus it would appear that in this sample the bias introduced by culling resistant cows is not great.

Table 3. *Udder infection and age effect in quarters of forty-two cows according to age in lactations*

Total new infections

Age group ...	1st	2nd	3rd	4th	Total
No. infected	23	50	66	66	205
No. in milk	167	167	167	167	668
% infected	13·8	29·9	39·5	39·5	30·7

$\chi^2 = 34{\cdot}77$. $P < 0{\cdot}01$.

Table 4. *Mastitis in infected quarters classified according to age in lactations and stage of lactation*

Month of lactation	1	2	3	4	5	6	7
First lactations							
No. of quarters with mastitis	8 (88·9)	8 (47·1)	1 (4·3)	4 (20·0)	5 (21·8)	4 (14·3)	3 (10·7)
No. of quarters infected	9	17	23	20	23	28	28
Second lactations							
No. of quarters with mastitis	12 (33·3)	9 (31·0)	7 (21·2)	11 (25·5)	3 (5·6)	8 (14·5)	3 (5·6)
No. of quarters infected	36	29	33	43	54	55	54
Third lactations							
No. of quarters with mastitis	16 (33·3)	3 (6·8)	5 (10·0)	2 (3·8)	6 (13·0)	3 (6·5)	6 (11·5)
No. of quarters infected	48	44	50	52	46	46	52
Fourth and over lactations							
No. of quarters with mastitis	45 (28·7)	11 (8·3)	8 (5·6)	19 (12·6)	18 (11·0)	18 (11·1)	20 (14·1)
No. of quarters infected	157	132	144	151	163	162	142
χ^2 value	14·15**	27·89**	9·21*	10·33*			

* Significant when $P < 0{\cdot}05$. ** Significant when $P < 0{\cdot}01$.
Per cent figures appear in brackets.

A further analysis was carried out, the results of which are given in Table 4 and Fig. 4. In this, mastitis in infected quarters has been classified for each month of lactation within each lactation age group. There are only limited data for each of the groups but there appears to be a difference in the lactation trends. In young cows a high proportion of infections cause mastitis in the first 2 months of lactation, a trend less pronounced in older cows. This aspect will be investigated in a later publication.

Discussion

There is general agreement amongst investigators that the incidence of udder infection increases with age (Bendixen (15); Klein & Learmonth (16); Murphy (17,18); Arthur (19); McEwen & Cooper (20); Seelemann (21); Schalm & Ormsbee (22); Schalm & Woods (23); Hughes (24)). Others have stated that both infection and mastitis incidence increase with age (Plastridge, Anderson, White & Rettger (25); Hopkirk, Palmer-Jones & Whittlestone (26); Ward & Castle (3); Withers (5)).

The results presented in this paper are in general agreement with those reported by previous workers in showing a relationship between age and the overall incidence of udder infection and mastitis, but our figures demonstrate that this increase with age is the

result of re-infections and persisting infections, and that first infections show no significant change with age of animal. Furthermore, the data show that the increase in mastitis with age is entirely due to there being more infection in older cows. The proportion of infections that give rise to mastitis show little change as cows become older.

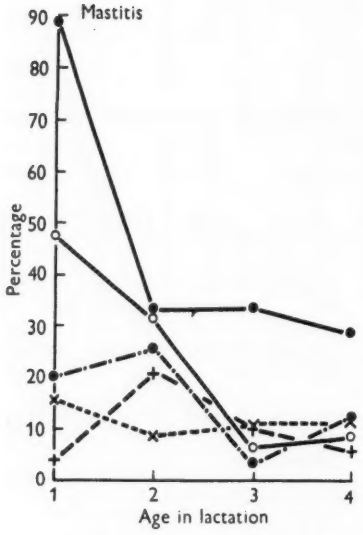

Fig. 4. Mastitis in quarters according to age and stage in lactations. ●, first month; ○, second month; +, third month; ⊙, fourth month; ×, fifth–seventh month.

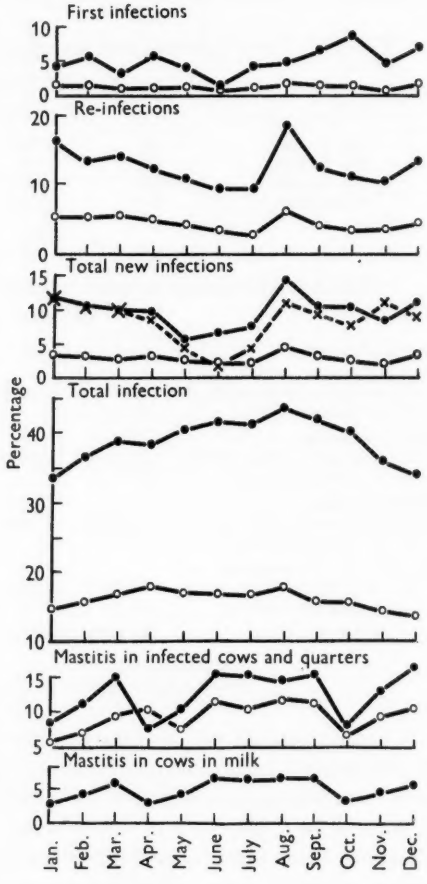

Fig. 5. Udder infection and mastitis in cows and quarters according to season. ●, cows; ○, quarters; ×, calving frequency.

Separate analyses have not been made for the different types of infection, but from the data presented in a previous paper (1) it will be seen that the rise in infection and mastitis with age applies to both streptococcus and staphylococcus infection. The latter finding is not in agreement with the reports of Murphy (17) and Ormsbee & Schalm (27).

Although from Fig. 2 it appears that, at least for quarters, the first infection rate declines with age, this trend is not statistically significant. A decline was to be expected because the most susceptible cows are probably infected in their first lactation. We would also expect second calving cows that had not previously been infected to have on the

average a greater *inherent* resistance than all first calvers. Similarly, the *inherent* resistance of third and fourth calving cows, that had not previously been infected, should be progressively higher still. That the decline with age was not significant may be the result of a counter-balancing factor associated with age which makes all animals, irrespective of their inherent degree of resistance, more susceptible to infection as they become older. Such a factor has not yet been demonstrated satisfactorily.

A further explanation of the apparent absence of an age trend in first infections is that they occur by chance and therefore may take place at any time in the life of the cow. The reported differences in inherent susceptibility (Johnson (28); Ward (29); Murphy, Pfau, Lepard & Bartlett (30); Dodd & Neave (13); Dodd & Foot (14); Howell, Pattison, Holman & Smith (31)) make this hypothesis difficult to accept.

The re-infection rate for cows was very high for all age groups, but as there were only nine cows that had been infected before the first lactations began, the data from the group cannot be representative. Apart from animals in their first lactation there is an increase with age in the re-infection rate for cows which is statistically significant. The high re-infection rate in all age groups was primarily responsible for the increase with age of total new infections and total infection. This was more apparent with the data from quarters (Table 2). It is not clear whether the number of previous infections is a major factor in the increased incidence with age, or whether this is caused by one or more of the following factors: age, degree of exposure (e.g. the number of adjoining infected quarters), duration of exposure or greater inherent susceptibility.

The effect of season of the year on the incidence of udder infection and mastitis

Method

It was found that there was insufficient data to analyse for seasonal effects separately for the groups of cows calving in each month of the year. As a result the data for all years and ages were grouped according to the month of the year, except that the records from the first month of calving of each cow were excluded. This was done in order to remove most of the bias caused by stage of lactation effects. The analysis was carried out for both cows and quarters for each of the various measures of infection and mastitis.

Results

In Table 5 and Fig. 5 the results of this analysis are presented. None of the trends for infection and mastitis of cows with season are statistically significant, and most of the variation can probably be accounted for as an incomplete removal of the stage of lactation effect and to the age differences of spring- and autumn-calving cows. In a previous publication (1) data were given of the calving frequency of this herd, and it is clear from tables 3 and 4 in that paper that there were marked seasonal trends in this frequency, and also that autumn-calving cows tended to be much younger than those calving in the spring months. These variations could account for the peak of first infections in October and of re-infection in January and February. Total infection rose gradually from January to August, and then declined to December. This trend might be associated with seasonal trends in the herd lactation age, which rose to a maximum in August and then declined as the young animals came into milk (1). The trends of mastitis incidence neither follow

Table 5. *Udder infection and mastitis in cows and quarters according to season*

Month of calving	Classification: Free from infection*	Classification: Previously infected	Classification: No. in milk	First infection: Infected	First infection: % of no. free from infection	Re-infection: Re-infected	Re-infection: % of previously infected	Total new infection: Newly infected	Total new infection: % of no. in milk	Total infection: No. infected (old, new and re-infections)	Total infection: % of no. in milk	Mastitis: Mastitis cases	Mastitis: % of total infection	Mastitis in cows in milk: No. with mastitis	Mastitis in cows in milk: % of no. in milk
							Cows								
Jan.	118	201	319	5	4·2	33	16·4	38	11·9	108	33·8	9	8·3	9	2·8
Feb.	135	207	342	8	5·9	28	13·5	36	10·5	125	36·5	14	11·2	14	4·1
Mar.	145	235	380	5	3·4	33	14·0	38	10·0	147	38·7	22	15·0	22	5·8
Apr.	155	253	408	9	5·8	31	12·3	40	9·8	156	38·2	12	7·7	12	2·9
May	151	274	425	6	4·0	30	10·9	36	5·5	172	40·5	18	10·5	18	4·2
June	137	260	397	2	1·5	24	9·2	26	6·5	166	41·8	26	15·7	26	6·5
July	122	236	358	5	4·1	22	9·3	27	7·5	148	41·3	23	15·5	23	6·4
Aug.	105	220	325	5	4·8	41	18·6	46	14·2	141	43·4	21	14·9	21	6·5
Sept.	108	228	336	7	6·5	29	12·7	36	10·7	141	42·0	22	15·6	22	6·5
Oct.	102	230	332	9	8·8	26	11·3	35	10·5	133	40·1	11	8·3	11	3·3
Nov.	105	212	317	5	4·8	22	10·4	27	8·5	114	36·0	15	13·2	15	4·7
Dec.	114	200	314	8	7·0	27	13·5	35	11·1	107	34·1	18	16·8	18	5·7
		χ^2 value		None of these values was significant at the 5% level											
							Quarters								
Jan.	1132	492	1278	19	1·7	26	5·3	45	3·5	189	14·8	11	5·8	—	—
Feb.	1205	512	1382	19	1·6	27	5·3	46	3·3	215	15·5	15	7·0	—	—
Mar.	1312	569	1525	13	1·0	31	5·4	44	2·9	255	16·7	24	9·4	—	—
Apr.	1406	621	1537	16	1·1	31	5·0	47	3·1	274	17·8	28	10·2	—	—
May	1469	691	1714	19	1·3	28	4·1	47	2·7	289	16·9	22	7·6	—	—
June	1361	689	1599	11	0·8	24	3·5	35	2·2	269	16·8	31	11·5	—	—
July	1238	646	1447	13	1·1	19	2·9	32	2·2	241	16·6	25	10·4	—	—
Aug.	1151	621	1335	22	1·9	38	6·1	60	4·5	238	17·8	28	11·8	—	—
Sept.	1195	632	1374	19	1·6	25	4·0	44	3·2	219	15·9	25	11·4	—	—
Oct.	1158	609	1388	19	1·6	21	3·4	40	2·9	217	15·6	15	6·9	—	—
Nov.	1120	551	1271	8	0·7	20	3·6	28	2·2	181	14·2	17	9·4	—	—
Dec.	1130	507	1274	19	1·7	24	4·7	43	3·4	176	13·8	19	10·8	—	—
		χ^2 value		Not significant		Not significant		22·89 $P<0·05$	—	Not significant		Not significant			

* Free from infection as for Table 1.

calving incidence trends nor those for total infection, and although the monthly variations observed were not statistically significant there was more mastitis in the summer as a whole than at other seasons of the year.

When infection and mastitis in quarters was examined the variations due to season were small. However, the total new infection rate was again highest in August, and in this case significantly so ($P < 0{\cdot}05$). In order to see whether this rise in the total new infection rate was entirely due to incompletely removed stage of lactation effects a further analysis was carried out (Table 6). The monthly new infection rate was calculated for the

Table 6. *The monthly new infection rate of cows calving in the months January to April*

		Month of calving					
		Jan.	Feb.	Mar.	Apr.	Total	%
Jan.	In milk	63	—	—	—	—	—
	New infections	10	—	—	—	—	—
Feb.	In milk	63	54	—	—	—	—
	New infections	6	6	—	—	—	—
Mar.	In milk	63	54	53	—	—	—
	New infections	6	4	5	—	—	—
Apr.	In milk	60	53	53	45	211	13·3
	New infections	7	6	10	5	28	
May	In milk	59	53	53	45	210	12·4
	New infections	4	6	7	9	26	
June	In milk	58	53	53	45	209	8·6
	New infections	4	5	6	3	18	
July	In milk	57	53	53	45	208	7·2
	New infections	3	2	3	7	15	
Aug.	In milk	51	51	52	45	199	14·1
	New infections	4	10	6	8	28	
Sept.	In milk	41	51	49	42	183	12·0
	New infections	5	9	4	4	22	

months of May to September for 211 cows calving from January to April. In April 13% of these cows contracted new infections, while in the following months the percentages were 12, 9, 7, 14 and 12 respectively. The expected decline with stage is seen up to July, but in August the rate rose, and even in September it was high. Thus it appears that the rise in August is not due to stage of lactation or seasonal calving trends.

Discussion

There is no published evidence that there is a true seasonal trend in udder infection and mastitis which is independent of age and lactation stage, or that infected cows are more subject to mastitis at particular times of the year. Arthur (19), Ineson & Cunningham (9) and Berger & Francis (32) suggested that the incidence of infection increased in late summer and early autumn, and a similar seasonal trend in mastitis was reported by Watts (33, 6) and Neave, Phillips & Mattick (34). A high incidence of mastitis in spring was reported from New Zealand by Ward (35, 36) and Ward & Castle (3), but the data of all these workers are biased by the high calving frequency at the times quoted both in Great Britain and New Zealand. On the other hand, Minett *et al.* (8) and Withers (5) in England, and Murphy (37) in America, could find no seasonal trends in their data.

The evidence in this report shows the difficulty in freeing data of this kind from stage of lactation effects. However, it is probable that season of the year has a very minor

effect on udder infection. In the herd examined a slight increase in infection rate occurred in August. Mastitis incidence was high in the period June to September and in the month of December.

SUMMARY

1. The detailed udder health records of 530 lactations have been analysed on a monthly and lactation basis, so that separate studies of the first infection rate, re-infection rate, total new infection rate and total infection and mastitis incidence for both cows and quarters could be made. These data have been used to study the relationship of stage of lactation, age and season of the year with udder infection and mastitis.

2. With advancing lactation there was a decline in the rate of first infection, re-infection, total new infection and in mastitis in infected cows and quarters.

3. In early lactation there was a marked tendency for infected cows and quarters to develop clinical symptoms.

4. During the last 4 months of lactation there was no significant rise either in infection or in mastitis.

5. There was an increase in infection and mastitis with advancing age, but infected older cows were not more liable to develop clinical symptoms than younger cows.

6. There was an indication that the longer a quarter was free from infection the greater were its chances of remaining so.

7. No major seasonal trends were found in the incidence of udder infection and mastitis, although the incidence was slightly higher in the summer months.

We wish to acknowledge the advice and encouragement of Dr A. T. R. Mattick and Mr A. S. Foot. Our thanks are due to Mr C. Machin for preparing Figs. 1–5 for publication, and to all of our colleagues who have assisted us.

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624. UDDER INFECTIONS IN THE 'DRY PERIOD'

II. THE EFFECT OF WITHDRAWING SECRETION FROM THE DRY UDDER ON THE INCIDENCE OF INFECTION

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A high new infection rate in the udders of dairy cattle in the early dry period has been shown by Neave, Dodd & Henriques (1). One criticism of this study is that the sampling of the udder secretion necessary for the investigation breaks the natural seal, which is believed to be formed in the teat orifice of dry cows as a barrier against infection. To find out whether dry quarters that are sampled in the early dry period are more prone to infection than unsampled quarters, and at the same time to measure the importance of the so-called natural seal, the following experiment was carried out.

THE HERDS

Two *Streptococcus agalactiae*-free herds, Da and Sk, were used, and these provided seventy-five cows for dry-period study during 1949–50. Herd Da was an Ayrshire herd milked in a parlour by a machine fitted with a moulded liner. Herd Sk was a British Friesian herd milked by bucket plant using liners similar to herd Da; milking was carried out in two adjacent cowsheds, one containing a single and one a double row of standings.

METHODS

Foremilk samples were taken from each quarter in the last 2 weeks of lactation, and a third sample was obtained within 2–3 days of the last milking. After drying-off by intermittent milking, two quarters of each cow had 1–5 ml. of secretion drawn from them at weekly intervals for bacteriological examination, and then all four quarters were sampled in the 4th and 5th week of the dry period. Cows that were already infected in one quarter on drying-off had either one or two non-infected quarters sampled, while cows already infected in two quarters on drying-off had one infected and one non-infected quarter sampled during the first 3 weeks of the dry period. Animals with more than two infected quarters at the time of drying-off were not used. The experiment was confined to the first 5 weeks of the dry period since this has been found to be the time when most new infections occur (1). When results from the last two samples were dissimilar further samples were taken. The bacteriological methods used have been described by Neave *et al.* (1) and Oliver, Dodd & Neave (2).

RESULTS

The number of quarters examined and the numbers becoming infected in each group are shown in Table 1.

Each treatment was applied to 150 quarters. Of the sampled quarters seventeen became infected in the first 4 weeks of the dry period, while twenty-one new infections

appeared in the quarters that were not sampled until the fourth and fifth week. This difference was not significant.

The types of micro-organism causing infection are shown in Table 1. There were significantly fewer ($P < 0.05$) staphylococcus infections in the sampled quarters than in unsampled quarters, while the incidence of 'green' infections was the reverse, this difference was not significant.

Table 1. *The effect of sampling on the new infection rate in dry quarters*

Treatment	Total quarters	Quarters not infected	Quarters becoming infected	Micro-organisms* causing infection		
				Staph.	Green	Others
Sampling	150	133	17	5	10	2
No sampling	150	129	21	14	5	2
Total	300	262	38	19	15	4

* Staph. = *Staphylococcus aureus*. Green = *Streptococcus uberis* and Group D streptococci. Others = *Corynebacterium pyogenes* and *Bacterium coli*.

THE NUMBER OF NEW DRY-PERIOD INFECTIONS PERSISTING TO THE NEXT LACATION

There were thirty-eight quarters infected in the dry period and, of the cows calving again, twenty-five of these quarters were available for sampling. The new infections persisted in thirteen (52%) of the twenty-five quarters.

DISCUSSION

The results show that in these two herds there was no difference in the incidence of new dry-period infections in quarters that were sampled and those that were not. The use of a half-udder technique, by ensuring an equal susceptibility in each group, adds a much greater precision to an experiment of this kind than is suggested by the 300 quarters available for investigation. There appears to be little doubt that, provided careful sampling methods are used, no increase in the new infection rate occurs when small quantities of udder secretion are removed in the dry period. In fact, it would appear that sampling, or the disinfection of the teat orifice which precedes sampling, reduces the new infection rate with *Staphylococcus aureus*. This finding requires further study.

About 15% of quarters non-infected on drying-off became infected in the dry period, and 52% of these persisted until the next lactation. These data support previous findings (1), except that the number of quarters becoming infected was lower. This is probably due to environmental and physiological differences between the herds.

Of the factors protecting the mammary gland in the dry period it does not seem that a natural sealing of the teat orifice is important in preventing infection in the first few weeks of the dry period.

SUMMARY

1. An experiment was carried out with seventy-five dry cows to study the effect of taking samples of udder secretion for bacteriological examination on the incidence of new infection in the early dry period. The incidence of new infection in two quarters of each cow, sampled at weekly intervals after drying off, was compared with that in the remaining two quarters sampled in the fourth and fifth week of the dry period.

2. No difference in the new infection rates was found between the quarters that were sampled early in the dry period and those that were not.

3. Significantly fewer ($P<0{\cdot}05$) staphylococcus infections occurred in quarters sampled in the early dry period compared with those sampled at a later stage.

4. About half of all the new infections persisted until the next lactation.

5. It was concluded that if natural sealing of the teat orifice or teat sinus takes place it is of minor importance in preventing infection in the early dry period.

We wish to thank Dr A. T. R. Mattick and Mr A. S. Foot for their interest, Messrs A. E. W. Dance and P. L. d'E. Skipwith for the facilities provided on their farms and those who have given technical assistance.

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625. UDDER INFECTIONS IN THE 'DRY PERIOD'

III. THE METHOD OF DRYING-OFF COWS AT THE END OF LACTATION

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INTRODUCTION

In previous publications(1,2) it has been shown that in the early dry period the new infection rate in the udders of dairy cattle can be much higher than in the rest of the dry period or during lactation. Also the so-called 'natural seal', allegedly formed in the teat orifice on drying-off, is not a major factor preventing infection.

It is often recommended that dairy cows should be dry for at least 6 weeks between lactations. The methods by which lactation is ended vary greatly from herd to herd, but all are based on two distinct techniques.

The first consists of abruptly ceasing the normal daily milkings on the day chosen for the cow to go dry; this is here described as 'stop' milking. This method has support on physiological grounds, because when regular milking is discontinued internal udder pressure rises and within 48 hr. prevents further milk secretion. The udder contents are then gradually reabsorbed. Many farmers believe that the high internal udder pressure which may result from this method of drying-off predisposes the udder to mastitis, but it has been argued(3,4,5,6) that the udder is under stress for a short time only, and for this and the physiological reasons the method is the better.

In the second method of drying-off—by either omitting one milking each day, increasing the intervals between milking or by leaving the udder incompletely milked—the milk yield at the final milking is reduced. These methods can be referred to collectively as 'intermittent milking'. They have been advocated(7,8,9,10) because it is considered that the udder is not subjected to intense internal pressure and the risk of mastitis, therefore, would be reduced.

An experimental comparison of the effects on udder infection of the 'stop' and 'intermittent' methods of drying off has been reported by Neave, Dodd & Lee(11). Their experiment, using 100 cows, was designed so that two quarters of the udders of each cow were dried off by 'stop' and two by 'intermittent' milking. Early dry-period infection occurred in thirty-four of 170 non-infected quarters dried off by 'stop' milking and in twenty-one of 173 non-infected quarters dried off by the intermittent method. Quarters that were infected on drying-off were excluded. There were fifteen and nine staphylococcus infections in each group respectively. The treatment difference for total new infections was significant ($P < 0{\cdot}05$). An objection to this experimental design is that milking the quarters on the intermittent treatment may affect those on the 'stop' treatment. The milk production of the 'stop' quarters was probably stimulated by milking the other quarters, and also the intermammary pressure of the 'stop' quarters would show temporary increases when the other quarters were milked because milk ejection would take place.

The object of the experiment described below was to make a between-cow, as distinct from a within-cow, comparison of the 'stop' and intermittent methods of drying-off on the new infection rate in the early dry period.

EXPERIMENTAL METHODS

The experiment lasted for 2 years (1951–53), using 126 animals in the herds of the National Institute for Research in Dairying (Herd N). There were 188 dry periods for study from the 126 cows examined, and for convenience each dry period is treated as if it was from a different cow. Drying-off was begun 2 months before the animals were due to calve again or when the daily milk yield had dropped to 7 lb. In order to reduce age bias experimental treatments were allocated at random to four age groups of cows separately, fourth and over lactations being listed together.

The experiment was conducted in two parts. In the first year animals dried off by intermittent milking were milked once a day for 28 days and then milked again 3, 7 and 14 days later. Unfortunately, some of these animals became completely dry before the once-a-day milking period was completed. In the second year, therefore, the intermittent treatment was modified to once a day milking for 14 days followed by stripping out on the succeeding third and seventh days. Throughout the experiment 'stop' milking meant all milking was discontinued after the day allotted for the animals to go dry.

During lactation, foremilk samples for bacteriological examination were taken each 1–2 months, or more frequently. All animals going dry were sampled in the week before the final twice-daily milking and on the last day that this milking took place. In the first year cows on intermittent treatment were then sampled weekly for 6 weeks, and in the second year in the fifth and sixth weeks only. Thus in each year there were records for each cow of the fifth and sixth weeks following the end of twice daily milking. Cows on 'stop' milking were sampled in the fifth and sixth weeks after the final milking of lactation, and for both treatments further samples were taken if the results of the bacteriological tests of the fifth and sixth week samples were not in agreement. The dry udders were inspected and palpated for abnormality at this time. In addition, the udders of the animals on 'stop' milking treatment were inspected and palpated on the seventh and fourteenth day after the final milking to detect abnormal quarters. Grossly abnormal quarters were infused with antibiotics.

BACTERIOLOGICAL METHODS

Milk samples were taken after careful examination and thorough disinfection of the teat apex. The method of examination of the samples and assessment of infection have been described previously (1).

RESULTS

The numbers of animals used, the numbers on each treatment and the numbers in each part of the investigation are shown in Table 1. There were 188 animals available for sampling in the fifth and sixth weeks of the dry period; ninety-one animals were dried off by 'stop' milking and ninety-seven by intermittent milking, and of these ten and eleven, respectively, were sold after they had been dry for 6 weeks.

The results from the two treatments were examined with respect to the numbers of

cows and quarters showing new infections and the type and severity of the infections. The results of Parts 1 and 2 of the experiment were similar and are thus grouped together. New infections have been recorded separately for cows that were free from infections in all quarters on becoming dry, and for cows that were already infected in one or more quarters. Non-functional quarters have been excluded from the analysis. The results are presented in Table 2.

Table 1. *The number of animals on experiment*

	Stop		Intermittent	
	Calving after treatment	Sold dry after treatment	Calving after treatment	Sold dry after treatment
Part I	37	5	45	3
Part II	44	5	41	8
Total	81	10	86	11
All cattle	91		97	
Mean age	2·8 lactations		2·7 lactations	

Table 2. *The new infection rate and the occurrence of abnormal secretions in cows and quarters according to the method of drying off*

	Animals that were not infected on drying-off				Animals that were infected in one or more quarters on drying-off				Total			
	Cows		Quarters		Cows		Quarters (non-infected)		Cows		Quarters (non-infected)	
Method of drying-off*	Stop	Inter	Stop	Inter	Stop	Inter	Stop	Inter	Stop	Inter	Stop	Inter
No.	56	58	223†	232	35	39	82	94‡	91	97	305	326
Becoming infected	21	15	44	27	20	27	31	33	41	42	75	60
Percentage infected	37·5	25·9	19·7	11·6	57·1	69·2	37·8	35·1	45·1	43·3	24·6	18·4
No. showing abnormal secretions	11	9	18	10	6	12	9	13	17	21	27	23
Percentage abnormal	19·6	15·5	8·1	4·3	17·1	30·8	11·0	13·8	18·7	21·6	8·9	7·1
Mean lactation age	2·2	2·1	—	—	3·9	3·7	—	—	2·8	2·7	—	—

* 'Stop' = abrupt cessation of milking. 'Inter' = intermittent milking.
† One blind quarter excluded.
‡ Two blind quarters excluded.

The total number of dry-period infections

New infections occurred in 41 (45·1%) of the 91 cows dried off by 'stop' milking and in 42 (43·3%) of the 97 cows dried off by the intermittent method. The corresponding figures for quarters were 75 (24·6%) of 305 and 60 (18·4%) of 326. Abnormal secretion was recovered from 17 (18·7%) of 91 cows and 21 (21·6%) of 97 cows on 'stop' and intermittent methods of drying-off, respectively, and the corresponding figures for quarters were 27 (8·9%) of 305 and 23 (7·1%) of 326 for the two treatments respectively. None of these treatment differences is significant.

New infections in quarters of *cows* not infected when dried off, however, were significantly more numerous ($P < 0·05$) in the 'stop' than in the intermittent milking group, being 19·7 and 11·6% respectively. These data, from cows not infected on drying off, were further divided into those that had not a previous history of infection, and those

known to have been infected. The results (Table 3) show that of the cows previously infected, 45·3% contracted new dry-period infections, while the corresponding figure for cows not previously infected was 19·7%. These figures show a significant difference ($P < 0{\cdot}01$).

A significantly higher ($P < 0{\cdot}01$) proportion of new infections occurred in cows that were already infected in one or more quarters than in cows free from infection on drying-off (Table 2).

Table 3. *The effect of previous infection on new dry period infection in cows that were not infected when dried off*

	No previous infection	Previously infected
No. of cows free from infection on drying off	61	53
No. of cows with new dry-period infections	12 (19·7%)	24 (45·3%)

The persistence of dry-period infections

When the dry periods studied were complete, and the cows calved, milk samples were taken on the day of calving and during the first 14 days of lactation. The persistence of dry-period new infections is shown in Table 4. About 50% persisted to calving and about 30% showed clinical symptioms of disease. These results are in general agreement with Neave *et al.* (1).

Table 4. *The proportion of new dry-period infections persisting until calving and the number with mastitis at this stage*

	New infections in dry period*	Infections present at calving	
		No.	With mastitis
Stop	66	30 (45·5%)	12 (40·0%)
Inter	48	28 (58·3%)	7 (25·0%)
Total	114	58 (50·9%)	19 (32·8%)

* Excluding infections that were treated and cured in the dry period and also infections in cows sold before calving.

The effect of method of drying-off on existing infections

The effects of the experimental treatments on infections present at drying-off are presented in Table 5. Three-hundred and sixty-four quarters were dried off by 'stop' and 388 by intermittent milking, and of these, 58 and 60 quarters respectively were already

Table 5. *The effect of the experimental treatment on the course of infections present at drying-off*

	Stop	Intermittent
No. of infected quarters	58	60
Contracting further infections	5	6
Old infections persisting to next calving	54	55
Percentage	93·1	91·7
Old infections in quarters showing mastitis	15	12
Percentage	25·9	20·0

infected when drying off began. The numbers of infected quarters contracting further infections, five and six respectively, are too small to draw conclusions. Old infections

persisted until the next calving in 54 (93·1%) and 55 (91·7%) of quarters respectively. The corresponding number of these quarters that had clinical symptoms of disease was 15 (25·9%) and 12 (20·0%). None of these differences is significant at the 5% level.

Types of infection in the dry period

Most of the infections in the dry period were caused by 'green' streptococci and staphylococci, and although five coliform infections were found, they occurred in association with other micro-organisms. The type of pathogen causing dry-period infections is

Table 6. *The type of new infection in cows*

	Stop			Intermittent		
Type of micro-organism*	Cows not infected at drying-off	Cows infected at drying-off	Total	Cows not infected at drying-off	Cows infected at drying-off	Total
Staph.	6	5	11	2	10	12
Green	9	8	17	5	12	17
Mixed	5	7	12	8	5	13
C. py.	—	1	1	—	—	—
Total	20	21	41	15	27	42

* Staph. = *Staphylococcus aureus*. Green = *Streptococcus uberis*, *Str. dysgalactiae* and Group D streptococci. C. py. = *Corynebacterium pyogenes*. Mixed = two or more of the above micro-organisms.

Table 7. *The type of new infection in quarters*

	Stop			Intermittent		
Type of micro-organism*	Quarters of cows not infected on drying-off	Quarters of cows infected on drying-off	Total	Quarters of cows not infected on drying-off	Quarters of cows infected on drying-off	Total
Staph.	17	10	27	7	14	21
Green	23	13	36†	16	17	33†
Mixed	2	8	10	4	2	6
C. py.	2	—	2	—	—	—
Total	44	31	75	27	33	60

* See Table 6 for key.
† Four of thirty-six and six of thirty-three green infections were caused by *Streptococcus dysgalactiae*.

shown in Table 6 (cows) and Table 7 (quarters). The distribution of types of micro-organism infecting each group of animals was very similar and no differences could be associated with method of drying-off.

The effect of method of drying-off on milk yield in the lactation following the dry period studied

Milk yields were available for lactations preceding and following the dry periods studied for seventy-five and eighty-five animals dried off by 'stop' and intermittent milking respectively. The data are presented in Table 8. There was a decline in yield in the

Table 8. *The effect on yield of method of drying off*

Method of drying-off	No. of cows	Lactation before going dry (gal.)	No. of cows	Succeeding lactation (gal.)	Decline (%)
'Stop'	75	8154·6	75	7404·9	9·2
Intermittent	85	7980·6	85	7117·6	10·9

lactations which followed the dry periods studied, and this was similar for each group of animals, i.e. 9·2% for 'stop' and 10·9% for intermittent. There is clearly no treatment difference, and the decline is probably a result of various experiments adversely affecting milk yield that are carried out in the herd.

DISCUSSION

The data collected from the herds, when taken as a whole, suggests that the method of drying-off does not have a major effect on the new infection rate of the dry period. In the case of cows that were not already infected at the time of drying off, there was significantly more infection ($P < 0{\cdot}05$) when the lactation was terminated abruptly, a finding in agreement with Neave *et al.* (11). The mean age in lactations for the cows not infected on drying-off was 2·2 and 2·1 for 'stop' and intermittent milking respectively, while the comparable mean age of the cows that were infected on drying-off was 3·9 and 3·7. It may be that a slightly increased infection rate associated with 'stop' milking was masked in the older animals (i.e. cows infected on drying-off) because the increased incidence of infection associated with age (1:12) was of more importance than the method of ending lactation.

There was a difference in the incidence of new infection among the cows that were not infected on going dry irrespective of the method of drying-off. Cows with a previous history of infection contracted more than twice as much infection as cows that had not been infected before. The difference was highly significant ($P < 0{\cdot}01$), and further demonstrates that once a cow has contracted an infection she is more liable to be re-infected (12).

The total infection rate for all cows was very similar to that found by Neave *et al.* (1). New infections were present in about half of the dry periods studied, about half of these persisted until calving, and about a third of these persisting infections caused clinical symptoms (Table 4).

There is no obvious explanation for the higher new infection rate in the early dry period of the younger animals dried off by 'stop' milking, although it confirms the earlier work of Neave *et al.* (1). It is possible that these younger animals have smaller udder capacities and a higher persistency of lactation. Thus higher internal udder pressure may be built up after the last milking, which may be sufficient to open the teat orifice and allow milk to leak out. If this occurs the access into the udder of bacteria on the skin of the teats might be facilitated.

Palpation of the udders of 'stop' milking animals, 7 and 14 days after they were dried off, may have caused contamination of the skin of the udder, but as the teats were rarely touched it is unlikely that palpation contributed to infection.

Although the overall infection rate for the groups of cows dried off by the two methods was similar, 45·1% for 'stop' and 43·3% for intermittent milking, it must be remembered that this experiment was carried out with cows yielding relatively small amounts of milk at drying-off. The effect of drying-off by 'stop' milking on infection in cows giving higher yields is not known.

Animals that were already infected, in one or two quarters, when dried off, developed more than twice as many infections in the dry period as animals free from infection at this time. This is in agreement with our previous findings for dry cows (1). The average

age of the 'already infected' group was much higher (Table 2), and the larger number of infections in this group may have been due to greater exposure to infection from adjoining infected quarters or greater susceptibility as a result of age, previous infection or inherent characters.

SUMMARY

1. An experimental study was made of the effect of drying-off by 'stop' or intermittent milking on the incidence of new infection in the early dry period of dairy cows. Ninety-one and ninety-seven dry periods were examined following the treatments, and the results were analysed on a cow and quarter basis.

2. The incidence of new infection was similar for groups of cows dried off by 'stop' or intermittent milking.

3. The quarters of cows that were not infected on drying-off showed a significantly higher incidence ($P < 0{\cdot}05$) of new infection following 'stop' than following intermittent milking.

4. Of the cows that were not infected at the time of drying off, those which had a previous history of infection showed a significantly higher ($P < 0{\cdot}01$) incidence of new dry-period infection than cows that had not been infected before.

5. There was a much higher incidence of new infection in cows that were already infected on drying-off compared with cows free from infection at that time.

6. About half of all the new dry-period infections persisted until the next lactation, and about one-third of these resulted in mastitis in the affected quarter.

7. The method of drying-off had no effect on infections that were already established in quarters going dry.

8. The types of pathogen causing infection were similar for both methods of drying-off.

9. The method of ending lactation did not affect milk yield in the lactation following the dry period studied.

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626. UDDER INFECTIONS IN THE 'DRY PERIOD'

IV. THE RELATIONSHIP BETWEEN THE NEW INFECTION RATE IN THE EARLY DRY PERIOD AND THE DAILY MILK YIELD AT DRYING-OFF WHEN LACTATION WAS ENDED BY EITHER INTERMITTENT OR ABRUPT CESSATION OF MILKING

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INTRODUCTION

In most dairy herds little difficulty is encountered in ensuring that cows are dry for the recommended 6 weeks between lactations. Some animals, however, particularly in high-yielding herds, are very persistent milk producers and may be giving several gallons of milk each day at the time when drying-off should begin. The work of Neave, Dodd & Henriques(1) has demonstrated the importance of the new infection rate in the early dry period. Little is yet known of the relationship between level of production when drying-off begins and of the method of drying-off itself on the incidence of dry-period infection. Recently, Oliver, Dodd & Neave(2) have compared the effect of the so-called 'stop' and intermittent methods of drying-off on the level of infection in dry cows. No major differences were found between the new infection rates of the animals dried off by these methods, but most of the cows were giving less than 7 lb. daily when dried off.

The study reported below was carried out in a herd where about a fifth of the cows were producing over 20 lb. of milk at the time when drying-off began. Data were collected to find out if the level of production at the end of lactation affects the new infection rate in the early dry period.

THE HERD

The heard (Cr) in which this study was carried out has been previously described by Neave, Higgs, Simpkin, Oliver & Dodd(3), in an experimental study of the relationship between mastitis and the method of stripping after machine milking. After August 1952, treatment with antibiotics was confined to clinical cases reported by the farmer, both in lactation and dry period. Most lactation infections were caused by *Streptococcus agalactiae* and a few by *Staphylococcus aureus*. About two-fifths of the cows were hand-stripped after machine milking, and the remainder were stripped by machine.

The average lactation production of milk for cows and heifers was about 1000 gal. with a fat content of 4%. Milking was carried out with bucket units fitted with extruded teat cup liners, and the machine was operated at a vacuum of 15–17 in. of mercury. There were about thirty-eight cows in the herd, which was milked in normal cowsheds.

METHOD OF DRYING-OFF

Drying-off was arranged so that cows were dry for at least 8 weeks. Each animal to go dry by 'stop' milking was thoroughly milked out at an evening milking and then for 48 hr. food was restricted to a little hay and water only. After this period the cow was

milked for the last time and allowed to run with the milking herd. Animals that had a long calving interval were milked once daily for a few weeks, and were thus dried off by intermittent milking. These comprise animals that were giving less than 7 lb. daily on drying-off. Stripping out after the last milking was by the method used in the normal milking of each cow in lactation.

METHODS OF DIAGNOSIS AND INTERPRETATION OF THE RESULTS

The bacteriological and sampling methods have been described in previous publications (1,3). The cows had been sampled regularly in lactation, and for the purposes of this study additional milk samples were taken in the week before going dry and at the final milking before drying-off. Dry cows were sampled again in the fourth and fifth weeks of the dry period, and in succeeding weeks if the results obtained from these two samples were not in agreement.

A quarter was judged to be free from infection if mastitis pathogens were not isolated from the two final samples of milk taken before drying-off. Where pathogens were found in the final lactation samples, although previous samples had been negative, the quarter was judged to be positive on this single test. Usually this diagnosis was confirmed by the subsequent dry-period samples. The presence of teat orifice erosions or sores at the orifice occasionally led to the recovery of small numbers of pathogens from the final milk samples. These were regarded as contaminants if the subsequent dry-period samples were free from these bacteria. All doubtful cases were excluded from the analysis as were quarters that were treated with antibiotics soon after going dry.

Streptococci other than *Streptococcus agalactiae* produced greening or browning of aesculin calf blood agar, i.e. *Str. dysgalactiae*, *Str. uberis* and some unidentified streptococci. For convenience these are together referred to as 'green' streptococci.

A quarter was judged to have been infected in the early dry period if: (*a*) similar pathogens were isolated from the fifth and sixth week samples; (*b*) pathogens were found in a sample from a clinically affected quarter; or (*c*) large numbers of pathogens were recovered from a single sample. There were ten cases in the latter group, and all were based on bacterial counts of over 1000 bacteria per ml. of secretion.

RESULTS

The dry periods of 113 cows were studied during the period August 1951 to January 1955. There were sixty-two cows that contracted new infections and 108 quarters were affected out of a total of 396 that were not infected when dried off.

The relationship between mean daily yield at drying-off and the infection rate in the early dry period

In Table 1 the number of new infections occurring in the early dry period is tabulated for both cows and quarters according to the daily yield of the cow at drying-off. It will be seen that the new infection rate in the early dry period increases with yield at drying-off. Forty-one per cent of the cows giving less than 7 lb. of milk daily became infected in the early dry period in 18% of the uninfected quarters. For cows giving over 21 lb. the corresponding figures were 71 and 43%. The increases were significant only for the data analysed on a quarter basis ($P < 0{\cdot}01$).

It has been demonstrated that there is a higher incidence of infection with old than with young cows (4), and this is also true for infection in the dry period (1). Thus the data were analysed to see if there was an age bias. Table 1 indicates that the groups of cows with the lowest new infection rate were, in fact, the groups with the highest mean lactation age. It is clear, therefore, that the higher incidence associated with higher yield at drying-off is not a reflection of age differences. In fact, if it had been possible to correct for the variations between the ages of the four groups, greater differences between the results would probably have been found.

Table 1. *New dry-period infection in cows and quarters for cows grouped according to daily yield in week of drying-off*

Mean daily yield in last week of lactation (lb.) ...	0–7	7¼–14	14¼–21	21¼+	Total
Total cows	48	28	23	14	113
Cows becoming infected	20	17	15	10	62
Percentage infected	41·7	60·7	65·2	71·4	54·9
Total quarters*	163	94	86	53	396
Quarters becoming infected	30	29	26	23	108
Percentage infected	18·4	30·9	30·2	43·4	27·3
No. of infected cows that subsequently calved	16	9	8	3	36
No. of infected cows still infected at calving	8	3	5	1	17 (47·2%)
No. clinical at calving	4	2	2	1	9 (52·9%)
Mean lactation age	4·2	4·0	2·4	2·8	(mean) 3·6

* Total quarters = number of quarters that were not infected when dried off.

Types of infection in the dry period

Examination of Table 2 reveals that more than half (fifty-eight) of the new dry-period infections were caused by 'green' streptococci, twenty-one by *Str. agalactiae*, fifteen by *Staphylococcus aureus* and fourteen were of mixed origin or caused by other micro-organisms. About 86% of the 'green' infections were caused by *Str. uberis*. It is interesting

Table 2. *Type of micro-organism causing new dry-period infection in quarters*

Group (lb.)	0–7	7¼–14	14¼–21	21¼+	Total
Type of micro-organism					
Staphylococcus aureus	4	5	5	1	15
Str. agalactiae	2	5	4	10	21
'Green'*	20	15	14	9	58
Other*	4	4	3	3	14
Total	30	29	26	23	108

* 'Green' = *Streptococcus dysgalactiae*, *Str. uberis* and Group D streptococci. Other = *Corynebacterium pyogenes* and *Bacterium coli*.

to note that the proportion of staphylococcus and 'green' streptococcus infections showed no apparent relationship to the milk yield of the cows at drying-off. The increase in the total new infection rate with yield shown in Table 1 is almost entirely due to an increase in *Str. agalactiae* infections which rose from 1·2% of 163 quarters in the low yield group to 18·9% of fifty-three quarters in the highest yield group. In this latter group only one of the ten infections with *Str. agalactiae* occurred in a cow already infected in one quarter when dried off. Furthermore, nine of the infections took place in cows that had calved once or twice only.

The detection of clinical cases in the dry period

About 40% of the quarters contracting new infections in the early dry period showed clinical symptoms before the following calving (Table 3). This did not appear to be related to milk yield at drying-off. A high proportion of the clinical cases was observed by the regular cowmen because the dry cows ran with the milking herd and were tied

Table 3. *The number of quarters with new dry-period infections showing noticeable clinical symptoms in the dry period*

Group ...	0–7 (lb.)	7½–14 (lb.)	14½–21 (lb.)	21½+ (lb.)	Total
New infections in the dry period	30	29	26	23	108
No. showing abnormality	11	9	14	9	43
Percentage abnormal	36·7	31·0	53·8	39·1	39·8
No. of abnormal quarters treated	2	8	11	6	27
Percentage of abnormal quarters treated	18·2	88·9	78·6	66·6	62·8

up and inspected twice daily. Inflammation of the affected quarter was usually the first noticeable symptom, and further examination often revealed other abnormalities, e.g. the quarter was indurated, hard or tender or the secretion was abnormal. When a clinical case occurred, samples were taken before the quarter was infused with penicillin.

The number of new dry-period infections persisting to the next lactation and the number of those becoming clinical

Of the 113 cows with dry periods available for study, 102 remained with the herd and calved again. Table 4 shows the number of new dry-period infections found in the non-infected quarters of the 102 animals, the type of micro-organism causing infection, the

Table 4. *The number of new dry-period infections persisting until calving and the number clinical at calving*

	Staph. aureus	*Str. agalactiae*	'Green'*	Other*	Total
No. of new infections†	12	5	35	7	59
No. of new infections present at next calving	5	3	9	3	20 (33·9%)
No. of new infections present at next calving and clinical	3	1	2	3	9 (45·0%)

* Key as for Table 2.
† Excluding quarters treated in dry period and those of cows sold.

number and type of infections persisting until calving and the number of quarters showing definite signs of disease. Data from quarters that were treated during the dry period were excluded.

After treatment of clinical quarters and the spontaneous recovery of others from infection, there were only 33·9% of the new dry-period infections present at the next calving, and 45% of these became clinical. These results are similar to those of Neave *et al.* (1), although the proportion of infected quarters that were still infected at the next calving was somewhat lower. This was due to the detection and treatment of a large proportion of clinically infected dry quarters (Table 3).

Yield in the next lactation

Comparison was made of the yield in the lactation preceding the dry period studied with that of the following lactation. Data for sixty-nine cows were available, and Table 5 shows the number of animals in each group with their total lactation yields before and after the dry periods studied.

Table 5. *The average lactation milk yield of the animals in the lactation preceding and following the dry period studied*

Mean daily yield on drying-off (lb.)...	0–7		7¼–14		14¼–21		21¼+	
No. of animals ...	22		21		14		12	
Lactation	Before	After	Before	After	Before	After	Before	After
Mean yield of group (gal.)	1104·0	1125·9	1074·2	1064·2	1065·7	1041·6	1167·0	1075·4

The results given in Table 5 indicate that at least in this sample the yield at drying-off did not affect the subsequent lactation yield. The differences between the two lactation yields of each of the four groups are not significant, neither is the correlation coefficient between the yield at drying-off and the difference between the two successive lactation yields.

The effect of the method of stripping after milking on new infection in the early dry period

The data were analysed so that a comparison could be made of the occurrence of new dry-period infection in quarters according to the method by which the cows were stripped out after machine milking. The results in Table 6 show that new dry-period infections were

Table 6. *New dry-period infection in quarters of cows that were hand- or machine-stripped in the lactation preceding the dry period studied*

Method of stripping in lactation preceding dry period studied	Number of new dry period infections					No. of quarters not infected at drying off	Mean age (lactations)	Mean yield on drying-off (lb.)
	Staph. aureus	*Str. agalactiae*	'Green'*	Other*	Total			
Hand	7	13	42	8	70	188	3·5	12·8
Percentage infected	3·7	6·9	22·3	4·3	37·2	—		
Machine	8	8	16	6	38	208	3·5	9·1
Percentage infected	3·9	3·9	7·7	2·9	18·3	—		

* Key as in Table 2.

more frequent in the quarters of cows that had been hand-stripped during lactation when compared with quarters of machine-stripped cows. The difference in the infection rates was statistically significant ($P < 0{\cdot}01$). This trend was apparent for both *Str. agalactiae* and 'green' infections. The numbers of staphylococcus and 'other' infections were too small to show an effect.

DISCUSSION

Abrupt cessation of milking has been advocated by Wayne, Eckles & Petersen (5), Wayne & Macy (6), Steyn (7) and Espe & Smith (8). On the other hand, Motion (9), Rose (10), Munch-Petersen, Murnane & Bull (11) and Johnson (12) advocate drying-off by intermittent milking. This method is most commonly used in practice.

The results presented in this paper indicate that for cows dried off by 'stop' milking the chances of a new infection occurring in the early dry-period increase with the daily yield of milk at drying-off.

The incidence of 'green' infections was not related to yield at drying-off, while the numbers of staphylococcus infections were too small to show definite trends. The overall increase associated with increasing yield on drying off is thus largely attributable to *Str. agalactiae* infections. The reasons for this occurrence are not apparent.

The results also indicate that the new infection rate is greater in cows that were hand-stripped in the previous lactation compared with those stripped by machine. Bacteria are easily spread by milkers' hands, and it is generally supposed that bacterial transfer is least if machine milking and machine stripping is practised. It has been shown (1) that most dry-period infections occur in the first 3 weeks after drying-off, and that *Str. agalactiae* is able to survive on teats for up to three weeks (13, 14). Thus the higher incidence of dry-period infection in hand-stripped cows may be caused by greater contamination of the teats before cows are dried off than is the case with machine-stripped cows.

There were insufficient data on latent infections to measure the effect of drying-off by abrupt cessation of milking.

The importance of regular inspection of the udders of dry cows is apparent (Table 3). In this herd the dry cows ran with the herd, and 40% of the new dry-period infections were detected by the cowmen and given treatment in the dry period. If these clinically affected quarters had not been found, milk yield in the following lactation would probably have been reduced since Crossman, Dodd, Lee & Neave (15) have shown the severe effects of clinical infections in the dry period and at calving.

On physiological grounds abrupt cessation of milking is thought to be the best way of ending lactation, because milk secretion ceases within 48 hr. However, the evidence in this paper shows that the incidence of udder infection and mastitis increases with increasing daily yield in the week before drying-off.

There was no major difference in the total lactation milk yield of the groups of cows (Table 5), and some cows were included in two or more of these groups. Yield at drying-off was partly related to length of calving interval, i.e. a long calving interval meant that a persistent milk producer was kept in milk until daily milking was no longer worth while. Thus cows giving relatively high yields at drying-off were not necessarily the greatest milk producers nor the fastest milkers, so that these factors (16, 17) cannot account for the high incidence of infection associated with high daily yield on drying-off.

Infection may be associated with the high internal udder pressure which will occur when abrupt cessation of milking is practised on animals giving relatively high milk yields. Internal pressure may be sufficient to open the teat orifice and allow milk to escape, an occurrence common with some lactating animals, particularly on lying down when udder pressure is greater still. If this occurs there would be a continuous film of milk from the teat sinus, through the streak canal to the exterior of the teat. Bacteria on the skin around the teat orifice would then have ready access into the teat sinus. If this is so there is no apparent reason why it should favour infection with *Str. agalactiae*. While the pressure developed at drying-off may not be so high as that developed in early lactation and during milking, it is maintained for a longer continuous period and may predispose to infection.

The practical implication of these results is the need to reduce the daily yield of milk before the last milking in lactation. Drying-off by intermittent milking achieves this end and results in the lowest dry-period infection rate. Where lactation is ended by abrupt cessation of milking, food which stimulates milk secretion should be excluded from the cow's diet and the diet restricted to small quantities of hay and water for a few days *before* drying-off is begun. Once milk production has fallen to about 10 lb. daily it appears safe to stop milking. On the other hand, it might be safe to stop milking at higher yields if pathogens could be removed from the skin of the teat at the end of lactation.

SUMMARY

1. Records were collected from a herd where many cows were producing relatively high daily yields of milk at the time of drying-off. Animals giving 7 lb. of milk or less daily were dried off by intermittent milking, and those giving over 7 lb. daily were dried off by 'stop' milking. The data from 113 dry periods were analysed so that a comparison could be made of the new infection rate in the early dry period according to level of production at drying-off.

2. The new dry-period infection rate was found to increase with yield at drying-off. This was not due to age differences between the groups of cows.

3. The rise in total infection rate with yield was due mainly to an increase in *Str. agalactiae* infections.

4. A high proportion of clinical quarters were detected in the dry period by the cowmen.

5. The yield in the lactation following the dry period studied was not related to yield at drying-off.

6. New dry-period infections were significantly ($P < 0{\cdot}01$) more numerous in quarters of cows that had been hand-stripped during lactation compared with quarters of machine-stripped cows.

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627. UDDER INFECTIONS IN THE 'DRY PERIOD'

V. THE EFFECT OF TEAT DISINFECTION AT DRYING-OFF ON THE INCIDENCE OF INFECTIONS IN THE EARLY DRY PERIOD

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INTRODUCTION

The high rate at which dairy cows contract udder infection in the early dry period has been clearly demonstrated (1,2,3,4). This new infection rate is greater than at any period in lactation, although only about half of the infections persist until calving. The work indicates the important need to control the new infection rate in dry cows, particularly as many of these infections become clinical at calving (5).

The reasons for the high incidence of early dry-period infection have not been established. It is probable that they are caused by pathogens which remain on the skin of the teats after the last milking of lactation, and then gain entry into the udder through the streak canal. The establishment of these infections is probably aided by the fact that milk removal has ceased, and there is also the possibility that the changes in the udder secretion which take place when a lactating udder goes dry may provide a better medium for bacterial growth.

This explanation of dry-period infection is supported by the evidence of Neave, Dodd & Lee (6), who showed that an infusion of 100,000 units of penicillin after the last milking caused a significant reduction in staphylococcus infections present at drying-off, but did not affect the number of new staphylococcus infections. If the latter had been present but undetected in late lactation then the new staphylococcus infection rate should have shown a reduction similar to that for infections present at drying-off.

There would appear to be three possible ways of preventing dry-period infection; to destroy all pathogens remaining on the skin of the teats after the last milking of lactation, to prevent any remaining bacteria from passing through the streak canal and finally to prevent the growth in the udder of bacteria that gain entry.

The work presented in this paper is an initial study of the value of skin disinfection on the incidence of new dry-period infections in seventy-nine animals.

THE HERD

The composition and management of the herd (N) has been described previously (7). It was machine milked and stripped by machine. During this study (July 1953—December 1954) the herd was free of *Streptococcus agalactiae*. Most udder infections were caused by *Staphylococcus aureus* and streptococci that cause greening or browning of aesculin blood agar. The latter comprise mainly *Str. uberis* but also *Str. dysgalactiae*, group D streptococci and a few unidentified streptococci. For convenience all these are referred to as 'green' streptococci.

METHODS

Cattle were dried off by abrupt cessation of milking either when the weekly milk yield had fallen to 56 lb. or less, or approximately 60 days before the next calving date. Animals that were infected in three or four quarters or had serious wounds or sores on the teats were excluded.

On the basis of lactation and pre-drying off tests the animals were classified as 'Staphylococci infected', '"Green" streptococci infected' and 'non-infected'. Cows with a mixed staphylococcus and streptococcus infection or two separate quarters infected with these micro-organisms were classified with the 'Staphylococci infected' group. Alternate animals were then allocated to experimental treatment (teat disinfection) and control (no treatment), with the exception that approximately equal numbers of cows with teat orifice erosions and teat sores were assigned to each group.

When the last milking of lactation had been completed the udders of cows on experimental treatment were thoroughly washed with a cloth dipped in sodium hypochlorite solution (800 p.p.m. available chlorine). After drying, each teat was immersed for 20 sec. in a 5% iodine tincture contained in a small cup. This was repeated 24 hr. later, and the animal was then turned out with other dry cows. The udders of control cows were not washed after the final milking. Dry udders were not handled again until 3 weeks after drying-off. There were a few severely clinical cases which necessitated udder handling, and these were excluded from the experimental results.

The methods by which infections had been determined while the cows were lactating have been described previously (7). Pre-drying off samples were taken in the week before and on the day of final milking. At sampling time careful note was made of teat orifice erosions, cuts and sores on the teat and other skin abnormalities which might influence the effectiveness of the treatment. Samples of secretion from each quarter were collected on the 21st and 28th day of the dry period.

RESULTS

The main results of the experiment are given in Table 1, where the data are classified for both cows and quarters. Cows that were not infected on drying-off showed clearly less infection when teats were disinfected with iodine.

The proportion of cows becoming infected in the experimental and control groups were 48·7 and 72·5%, while the corresponding figures for quarters were 23·2 and 31·2%. Both of these treatment differences are significant ($P < 0{\cdot}05$). Disinfection had no effect on the new infection rate of the non-infected quarters of cows that were already infected on going dry. If the results from these infected cows are excluded the difference in the infection rate for the remaining cows and quarters is highly significant ($P < 0{\cdot}01$).

THE TYPE OF PATHOGEN CAUSING INFECTION IN QUARTERS

The new infections in quarters were classified according to the type of micro-organism responsible (Table 2). The total *infections* were 36 in 138 and 56 in 141 quarters for the experimental and control groups respectively. This difference is significant ($P < 0{\cdot}05$).

It is apparent that teat disinfection exerted no influence on the new infection rate with 'green' streptococci, while there was a considerable treatment effect on the infection rate

Table 1. *The effect of teat disinfection on the new infection rate in cows and quarters during the early dry period*

	Experimental (teat disinfection)				Control (no treatment)			
		Cows		Quarters		Cows		Quarters
Pre-dry period classification of cows	Mean age lacts.	No.	Becoming infected	Not infected on drying off	With new infections	Mean age lacts.	No.	Becoming infected	Not infected on drying off	With new infections
Cows not infected	3·3	25	8	100	15	3·4	25	18	100	28
Percentage	—	—	32·8	—	15·0	—	—	72·0	—	28·0
Cows with *Staphylococcus aureus* infections	4·0	9	6	23	7	4·0	11	7	29	10
Cows 'green'* streptococci infected	4·2	5	5	15	10	4·5	4	4	12	6
Total cows infected on drying off	—	14	11	38	17	—	15	11	41	16
Percentage	—	—	78·6	—	44·7	—	—	73·3	—	39·0
Total	3·6 (mean)	39	19	138	32	3·7 (mean)	40	29	141	44
Percentage	—	—	48·7	—	23·2	—	—	72·5	—	31·2

* *Streptococcus uberis, Str. dysgalactiae* and Group D streptococci.

Table 2. *The type of micro-organism causing infection in quarters*

	Experimental (teat disinfection)				Control (no treatment)			
		Type of micro-organism causing infection in quarters				Type of micro-organism causing infection in quarters		
Pre-drying off classification of cows	No. of quarters not infected on drying-off	Staph.*	'Green'*	Other*	No. of quarters not infected on drying off	staph.*	'Green'*	Other*
Not infected	100	—	15	—	100	13	15	3
Staph.* infected	23	1	8	—	29	4	8	3
'Green'* infected	15	3	8	1	12	5	5	—
Total	138	4	31	1	141	22	28	6

* Staph. = *Staphylococcus aureus.* Other = *Corynebacterium pyogenes* and *Bacterium coli.* 'Green' = *Streptococcus uberis, Str. dysgalactiae* and Group D streptococci.

for staphylococci. New staphylococcus infections occurred in 4 of 138 and 22 of 141 quarters of the experimental and control groups, and this difference is highly significant ($P < 0{\cdot}01$). For quarters of cows that were not infected on drying-off, there were no staphylococcus infections in 100 disinfected quarters and 13 in 100 quarters not disinfected. This difference is again highly significant ($P < 0{\cdot}01$).

The cows that were infected on drying-off had 38 and 41 non-infected quarters in the experimental and control groups. These did not show treatment differences. Of the 59 'green' infections 55 (93·2%) were caused by *Str. uberis*.

DISCUSSION

The results show that teat disinfection, with 5% iodine tincture after the last milking of lactation, is effective in reducing the number of new infections in the following dry period. This is due to a significant reduction ($P < 0{\cdot}01$) in new infections caused by *Staph. aureus*, for there was no treatment effect on *Str. uberis*, while the number of *Str. dysgalactiae* infections were too few to show a treatment effect.

Previous work (6) has shown that the infusion of 100,000 units of procaine penicillin on drying-off is effective in reducing established infection by the common pathogens, but limits new infection with *Str. uberis* only.

Though easy to postulate, it is difficult to explain why teat disinfection appears to have had some control over new infections with *Staph. aureus* but not with *Str. uberis*, while penicillin had the reverse effect. This is especially so because of our observation (4) that *Str. uberis* infection occurred most frequently in cows that were hand-stripped, while there was no difference in the number of infections with *Staph. aureus* in machine- and hand-stripped cows. The explanation may lie in differences between the bacterial flora of the skin of the udder and teats in lactation and in the dry period. The main reservoir of *Str. uberis* is not known, but it can be isolated from the teats of pregnant heifers. On the other hand, while *Staph. aureus* also must occur sometimes in this site, we have been unable to recover it, although quarters infected with the micro-organism have been found (8).

It would appear that effective control of new and established dry-period infection may be achieved by a combination of udder infusion on drying-off followed by teat disinfection. Complete control has yet to be achieved but this should be nearer with the aid of a more persistent germicide and a more effective (or more persistent) antibiotic.

SUMMARY

1. The effect of disinfecting the skin of the teats at drying-off on the incidence of new infection in the early dry period has been studied. After the last milking of lactation the udders of alternate cows to go dry were washed with sodium hypochlorite solution (800 p.p.m.), dried, and each teat immersed in a 5% iodine tincture for 20 sec., and then again immersed 24 hr. later.

2. There was significantly ($P < 0{\cdot}05$) less new infection in cows that had teats disinfected than in control cows. This difference in infection was highly significant ($P < 0{\cdot}01$) for cows that were not infected on drying-off.

3. The treatment effect was due to a highly significant ($P < 0{\cdot}01$) reduction in the incidence of infection by *Staph. aureus*. New infection caused by *Str. uberis* was not affected.

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628. FURTHER STUDIES OF IMMERSION CLEANING OF MILKING EQUIPMENT

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Studies of the process of cleaning milking machines by immersing the equipment in caustic soda solution for the whole period between milkings have been continued for a further year. The previous results from eight commercial farms (Thiel, Clough & Clegg, 1955) showed the technique of immersion cleaning and the special milking and immersion equipment which had been evolved for the process to be practical and effective. But in two respects improvements were necessary before immersion cleaning could be recommended for general use. Calcium soap and inorganic calcium salts formed deposits, on the rubber and metal surfaces, which at monthly intervals had to be removed with acid. Moreover, rinse counts often gave total numbers of organisms of over 50,000/cluster, although milk samples were almost invariably satisfactory.

A preliminary experiment on one farm where ethylene diamine tetra-acetic acid (EDTA) was added to the caustic soda solution showed that this substance would prevent calcium deposits. Farm trials during 1955 were therefore designed to show the capabilities of EDTA on the eight farms which co-operated in last year's experiments. The Avon India Rubber Company kindly consented to manufacture liners containing bactericides, which it was thought might prevent high rinse counts, and small-scale trials of these liners have also been made.

TECHNIQUE OF IMMERSION CLEANING

All farms used the same strength of caustic soda and the same technique for cleaning the milking machine. Seven farms used Alfa Laval 20003-B extruded liners and one farm Alfa Laval hooded liners (24803-B). Another farm using Gascoigne Single Stretch liners was brought into the experiment (farm I). All liners were of natural rubber. The procedure of immersion cleaning at the farms may be summarized as follows:

Before milking. The basket containing the milking equipment was raised from the immersion solution and rested on the edge of the tank to drain. While this was taking place 5–10 gallons of water was run into the rinse trough and ½–1 oz. of approved hypochlorite solution added. The milking components were then transferred to the trough and rinsed free from caustic soda, after which they were assembled ready for milking.

After milking. The milking units were returned to the trough which still contained the rinse water used before milking. The stall tubes and milk tubes were removed from the lid and clawpiece, and placed one by one in the basket. The clawpieces were then opened, and the liners released from the teat cups which were slipped back to lodge on the special lugs moulded on the milk stems. The clusters were then placed on the bridge in the basket and the milking lids to the sides of the clusters. The basket and contents were lowered into the immersion solution in the tank and the lid replaced.

No special precautions were taken to rinse the equipment free from milk, since the rinsing resulting from the manipulations in the trough appeared to be adequate. However, both before and after milking, the long milk tubes were rinsed by running water through them from the cold-water tap.

At the end of each month the immersion tank was drained and rinsed out. It was then half-filled with water to which was added 3 lb. of flake caustic soda. Then 2, 4 or 6 oz. of EDTA were added and dissolved by stirring, the amount added depending on the hardness of the water, whether soft (less than 100 p.p.m.), hard (100–300 p.p.m.), or very hard (over 300 p.p.m.). The solution was then made up to normal level with water, the total volume being approximately 12 gallons.

On the day on which the solution was renewed the clusters were completely dismantled and the liners (Alfa Laval 20003-B) reversed and cut to normal length. If any deposits could be seen or felt these were removed by brushing in warm water.

The equipment used was the same as in the previous year (Thiel *et al.* 1955).

SAMPLING AND TESTS

A milk sample was taken fortnightly at each farm from the first can filled at a morning milking, iced, and tested within 3 hr. Contrary to the practice followed the previous year the can was not specially sterilized on the farm. The bacteriological results quoted are confined to keeping quality and initial colony count as measures of general milk quality, and initial presumptive coli-aerogenes tests as a measure of the efficacy of the milking technique. Keeping quality was determined by tests at 3 hr. intervals (after the first 24 hr.) on the milk incubated continuously at 22° C. until the clot-on-boiling end-point was reached. Presumptive coli-aerogenes tests were made in MacConkey's broth and colony counts by plating in Yeastrel milk agar; incubation was at 30° C. for 3 days for both tests.

To measure the bacteriological state of the milking equipment clusters were rinsed once a fortnight by passing 500 ml. of ¼ strength Ringer's solution 5 times through the cluster. The clusters were taken direct from the caustic soda solution for bacteriological rinsing, and to avoid the lethal effect of a high pH the Ringer's solution contained phosphate buffer. Plates were incubated for 3 days at 30° C.

RESULTS WITH USE OF EDTA

Effect on rinse counts and calcium deposition

The bacteriological data for June to September in Table 1 give an overall picture under summer conditions of the effectiveness of the cleaning technique when the caustic solution contained EDTA. The results from old and new liners may also be compared as the liners on most of the farms were renewed at the end of July. July and August were particularly warm months as may be seen from the atmospheric-shade-temperature figures given at the foot of Table 1.

On all farms EDTA successfully prevented calcium deposition. On farm F, where the water contained 360 p.p.m. total hardness, the use of 4 oz. of EDTA in the immersion tank prevented deposition for 3 weeks, but during the fourth week of the monthly cycle some accumulation of inorganic salts occurred. Subsequently 6 oz. of EDTA were used each month and no further deposition occurred. The water supplies on farms G and H

were relatively soft—less than 100 p.p.m.—and 2 oz. of EDTA prevented deposits from forming, although it was known from the previous year that plain caustic soda led to some deposition.

Table 1. *Bacteriological results for periods of 4 consecutive fortnights at various stages of liner life*

Farm		June–July					Aug.–Sept.		
		Extruded liners							
		Liners 1–8 weeks					Liners 1–8 weeks		
A	Keeping quality (hr.)	51+	48+	51+	51+	33	51+	51+	39
	Count (thousands/ml.)	1	3·2	0·2	2·3	29	3·3	8·2	7
	Coli, absent in (ml.)	10	10	10	10	0·01	10	10	10
	Rinse (thousands/cluster)	101	2·5	0·8	2·5	1·3	26	30	38
		Liners 9–16 weeks					Liners 1–8 weeks		
B	Keeping quality (hr.)	48	48+	51+	51	51+	51+	51+	51
	Count (thousands/ml.)	2·6	0·9	1·2	0·3	0·4	1·5	0·3	0·5
	Coli, absent in (ml.)	1	1	10	10	10	10	10	10
	Rinse (thousands/cluster)	0·2	3·5	0·2	1·5	1	1	1	<0·3
		Liners 9–16 weeks					Liners 1–8 weeks		
C	Keeping quality (hr.)	51	36	51+	51	51	45	51+	48
	Count (thousands/ml.)	0·6	6·4	3·9	2	9·2	37·6	1·6	10
	Coli, absent in (ml.)	10	10	1	1	10	0·001	1	1
	Rinse (thousands/cluster)	4·5	63	4	25	112	42	8	15
		Liners 9–16 weeks							
D	Keeping quality (hr.)	48	45	42	48				
	Count (thousands/ml.)	1·7	2·6	47	14		No samples		
	Coli, absent in (ml.)	1	10	0·001	0·001				
	Rinse (thousands/cluster)	14	27	9	9				
		Liners 9–16 weeks					Liners 1–8 weeks		
F	Keeping quality (hr.)	51+	48+	51+	51	51	36	42	51+
	Count (thousands/ml.)	0·2	0·5	1·7	1·1	0·3	0·6	1·0	1·0
	Coli, absent in (ml.)	10	10	1	0·1	10	0·1	0·01	10
	Rinse (thousands/cluster)	3	0·8	0·8	2·0	2·8	2	1·3	6·3
		Liners 9–16 weeks					Liners 1–8 weeks		
G	Keeping quality (hr.)	51	51	48	51+	51+	51	51	48
	Count (thousands/ml.)	0·4	1·8	1·3	0·6	1·5	0·6	0·6	1·5
	Coli, absent in (ml.)	10	10	10	10	10	1	10	10
	Rinse (thousands/cluster)	316	1·3	1·6	4·5	0·8	4	3	6
		Liners 1–8 weeks					Liners 9–16 weeks		
H	Keeping quality (hr.)	51+	39	51+	51	51+	51+	51+	51+
	Count (thousands/ml.)	1·2	2·9	13	11	2·3	3·4	4·8	3·2
	Coli, absent in (ml.)	10	1	1	10	10	10	10	10
	Rinse (thousands/cluster)	4·5	<0·3	1	2·5	0·3	1·3	1·7	6
		Moulded liners							
		Liners 9–16 weeks					Liners 17–24 weeks		
E	Keeping quality (hr.)	51+	48+	36	39	51	51+	48	51+
	Count (thousands/ml.)	0·5	7	37	51	0·4	0·5	3·4	0·9
	Coli, absent in (ml.)	1	10	1	0·001	1	10	10	10
	Rinse (thousands/cluster)	0·8	0·3	<0·3	0·3	0·3	2·8	2·0	0·8
		Liners 1–8 weeks					Liners 9–16 weeks		
I	Keeping quality (hr.)	51+	51+	51+	36	36	33	42	51+
	Count (thousands/ml.)	2·1	2·0	3·8	19	37	60	24	4·8
	Coli, absent in (ml.)	1	1	1	0·01	1	0·001	10	1
	Rinse (thousands/cluster)	190	0·5	16	27	20	6	10	6
Average shade temperature for 3 days around sampling date (° F.)		63·3	65·3	65·1	66·5	68·7	70·5	65·3	56·5

A comparison of the results for the same farms during August–November 1954 with the same period in 1955 shows that EDTA was instrumental in reducing the incidence of high rinse counts. In both periods liners were renewed at the end of July. The use of EDTA in 1955 reduced the incidence of counts over 50,000/cluster from 23% in 1954 (39 out of 171) to 6% in 1955 (5 out of 82). During 1955, colonies were picked from plates when the

counts were high. The organisms were mainly inert micrococci. The occasional occurrence of rinse counts over 50,000/cluster is therefore not considered sufficiently important to prevent commercial use of the process.

During 1955, the numbers of organisms recovered from clusters by rinsing were similar for liners in the first 2 months of use and for liners in the third and fourth months of use. Comparing results (Table 1) from farms where there are equal numbers of determinations for old and new liners, there are six counts over 10,000 for new liners and five for old liners out of a total of forty-eight counts. This emphasizes the beneficial effect of EDTA in preventing deposition of calcium salts, the previous year's results with plain caustic soda solution having showed much higher counts with old liners than new. Cluster-rinse counts were as good for July and August when the weather was particularly warm as for June and September when temperatures were lower.

Although EDTA prevented deposition of calcium soap and inorganic calcium salts, the liners were not always entirely free from deposits. On farms A, C, D and I the liners often felt slippery, particularly in the area of teat contact, after they had been rinsed free from caustic soda with cold water. As a rule some gelatinous material could also be seen in the region of the mouth ring. This material was probably sodium soap because it was soluble in water and could be made to froth.

On several of the farms rinse counts were invariably satisfactory (less than 50,000/cluster). Only on farms (A, C, D, and I) where there were detectable accumulations of gelatinous soap were high rinse counts found. It is also notable that on these farms the mouths of the liners were more liable to lose shape, the rubber being thickened as a result of fat absorption.

Effect on bacteriological quality of milk

The results for the summer period June–September inclusive shown in Table 1 confirm the previous year's finding that under the conditions of operation on these farms satisfactory milk was produced with equipment cleaned by the immersion process. The figures for keeping quality are satisfactory in all but two cases, a value at 22° C. of 36 hr. being taken as good and 42 hr. as excellent. In two instances values of 33 hr. were recorded; these were associated with abnormally high initial counts for the particular farms and also large numbers of coli-aerogenes organisms in the samples (farms A and I). It is reasonable to suppose that these organisms entered the milk from unsatisfactory milk cans or as a result of faults in milking technique. On a single occasion in September, two cans from each farm using immersion cleaning were examined bacteriologically. Of fourteen cans rinsed five showed total counts of over 5 million. There was no indication that the workers involved on these farms paid any attention at all to the condition of cans before filling them, and under these conditions bacteriological quality of the milk cannot be related to the bacteriological state of the milking equipment. If the few figures quoted for cleanliness of cans is any indication of what usually occurred on these farms it is rather surprising that the milk results were in fact generally satisfactory.

BACTERICIDAL LINERS

Assuming that high rinse-counts of clusters were related to the presence on the liners of a soap layer which protected bacteria from the caustic soda, it seemed that the incorporation of a diffusible bactericide in liners might prevent the growth of these bacteria.

A bactericide for this purpose must withstand the process of rubber manufacture without interfering with it, it must be sufficiently resistant to leaching to remain in the rubber after several months' immersion and yet be diffusible so that bactericidal action in the soap layer is appreciable. It is known that tetramethylthiuram disulphide (TMTD) when used in the rubber mix as an accelerator for the vulcanizing process results in rubber unsuitable for some laboratory purposes because it is bactericidal (Woiwood, A. J., Wellcome Research Laboratories, Beckenham, personal communication and Hirsch A., Lever Bros. Ltd., Sharnbrook, personal communication).

Four separate batches of liners containing bactericides were made for us by the Avon India Rubber Co. Ltd., to whom we are greatly indebted. The pattern of liners was 'Alfa Laval 20003-B'. The batches were:

(1) Containing TMTD, 0·7%.

(2) Containing QAC (Benzalkonium chloride, supplied by Gascoigne-Crowther Ltd., Reading, Berks), 1·85%.

(3) Containing QAC (Benzalkonium chloride, supplied by Cooper, McDougall and Robertson Ltd., Cooper Technical Bureau, Berkhamsted, Herts), 1·85%.

(4) Containing an organic bactericide selected by the Avon India Rubber Co. Ltd., and noted as 'X'.

In use the liners containing a quaternary ammonium compound behaved similarly to the controls; those containing compound 'X' became distorted at the mouth and appeared to lose elasticity much faster than normal liners; and liners containing TMTD were stiffer than normal liners and suffered from fatigue-splitting of the barrel after use for about 2 months. These liners were stiffer than normal because TMTD is a most effective accelerator for the vulcanizing process, but liners of suitable physical properties could probably be made if the mix composition were altered.

On farms using immersion cleaning where bactericidal liners were tried one cluster was equipped with one of the above bactericidal liners and the other cluster or clusters with normal liners. Three methods of test were used:

(1) After use on farms for 1, 2 and 3 months a short ring was cut from the mouthpiece end and placed on a Petri dish containing agar seeded with *Staphylococcus aureus*. The widths of zones of inhibition were measured after incubation at 37° C. for 2 days.

(2) At fortnightly intervals the milking machine clusters were rinsed five times with 500 ml. of sterile ¼ strength Ringer's solution and colony count determined (3 days at 30° C.).

(3) After 1, 2 and 4 months of farm use liners were swabbed using 20 ml. of sterile ¼ strength Ringer's solution and results reported as total numbers of organisms recovered. The mouthpiece end and the grab-ring end of each liner were swabbed separately, the mouthpiece end without disturbing the mouth ring and the grab-ring end after removing the grab-ring and milk stem.

A preliminary laboratory experiment using the zone-of-inhibition-test showed that all the experimental liners as delivered inhibited growth of *Staph. aureus*, but only the ones containing TMTD were also inhibitory to *Bacterium coli*. The zones of inhibition were widest with liners containing TMTD (about 10 mm.) and least with QAC-1 (1–2 mm.). All liners retained some if not all of their inhibitory power when stored in 1·5% caustic soda solution containing 0·25% EDTA for 52 days. Normal commercial liners from the batch used as controls in this work showed no inhibition.

The data in Table 2 show that of the four sorts of inhibitory rubber only the one containing TMTD retained in farm use appreciable power to inhibit *Staph. aureus*. Since it had already been shown that caustic soda alone did not destroy the inhibitory power, it seems that side reactions with other substances (fat, soap, protein) must account for the results obtained in farm practice.

Table 2. *Zones of inhibition after farm use of liners for* 1, 2 *and* 3 *months* (*test organism*, Staphylococcus aureus)

Bactericide	Width of zone of inhibition (mm.) after 1 month	2 months	3 months
TMTD	5	3	4
QAC-1	0	—	0
QAC-2	0·7	1	0·5
'X'	0	—	0

Table 3. *Rinse counts in thousands/cluster from bactericidal and control liners in farm use*

Date	TMTD	Control	Control	QAC-1	Control	QAC-2	Control	'X'	Control	Control
19 April	12	6	6	3	3	6	3	5	2	6
2 May	5	1	5	0·5	2	40	57	3	2	6
17 May	1	2	1	5	149	65	2	5	4	2
1 June	1	2	1	18	1040	76	15	8	2	10
12 June	1	935	12	176	38	4	5	0·5	<0·5	<0·5
30 June	<0·5	<0·5	4	4	2	120	8	0·5	1	10
12 July	<0·5	5	<0·5	1·5	<0·5	7	2	0·5	<0·5	<0·5
26 July	3	10	1	1	5	43	8	2	0·5	2
Proportion of high counts	0/8	1/8	0/8	1/8	2/8	3/8	1/8	0/8	0/8	0/8

Date	'X'	Control	TMTD	Control
8 Aug.	3	0	15	209
23 Aug.	20	32	6	79
7 Sept.	56	4	10	7
19 Sept.	8	69	7	23
4 Oct.	1	1	8	4
18 Oct.	22	5	14	98
1 Nov.	4	6	5	500
16 Nov.	20	72	11	9
Proportion of high counts	1/8	2/8	0/8	4/8

Horizontal lines in the table indicate when liners renewed.

In Table 3 are shown rinse counts in thousands/cluster for control and experimental liners in farm use. Counts are deemed to be satisfactory if they fall below 50,000/cluster. Data (Table 3 top) for liners containing TMTD and 'X' are inconclusive since high counts were obtained neither from experimental nor from control clusters. Results for liners containing quaternary ammonium compounds, although sparse, support the conclusion from inhibition tests which showed that the bactericidal activity soon disappeared with farm use. These liners were therefore omitted from the next series of tests, results of which are shown in the lower part of Table 3. In themselves the results are again inconclusive for liners containing substance 'X', but they tend to confirm the inhibition tests. Liners containing TMTD gave rinse counts which were strikingly better than their controls.

Confirmation of the usefulness of TMTD as a bactericide in rubber is given by the data in Table 4 for separate swabbings of the undisturbed mouthpiece end of liners and the

grab-ring end after dismantling. The numbers of organisms recovered from the mouthpiece end were usually low and were not affected by the presence of TMTD in the liners. At the grab-ring end, however, where the crevice between the liner and short milk-stem allows bacteria to grow undisturbed by the cleaning process, numbers were high for control liners and liners containing substance 'X', but were low with liners containing TMTD.

Thus of the four bactericides added to liners (TMTD, two closely related QACs and another organic bactericide noted as 'X'), only TMTD remained effective in farm use. Rubber containing TMTD gave excellent bacteriological results in this limited series of experiments, and further work is desirable. It is possible that rubber containing TMTD would be of advantage when used with methods other than immersion cleaning, for example, with components that are dry stored between milkings.

Table 4. *Numbers of bacteria recovered by swabbing separately the mouthpiece and grab-ring ends of liners after farm use. Liners were the same ones from which the data in the second part of Table* 3 *were obtained*

Type of liner	Time in use (months)	Liner no.	No. of organisms recovered from mouthpiece end (thousands)	No. of organisms recovered from grab-ring end (thousands)
TMTD	1	1	17	1
		2	0·9	0·2
Control		1	1·2	28,000
		2	0·1	25,000
TMTD	2	1	Not swabbed	1·4
		2	Not swabbed	0·4
Control		1	Not swabbed	45,000
		2	Not swabbed	34,000
		3	*Not swabbed*	*50,000*
		4	Not swabbed	3,900
TMTD	2	1	1·1	0·8
		2	0·6	1
		3	1·5	0·6
		4	2·7	0·2
Control	4	1	0·6	21,000
		2	8·1	47,000
		3	1	36,000
		4	0·7	30,000
'X'	1	1	404	7,500
		2	0·5	10,000
Control		1	0·8	50,000
		2	10,000	50,000
'X'	2	1	5,000	33,000
		2	390	41,000
Control		1	5	37,000

SUMMARY

Further studies of immersion cleaning have shown that no deposition of calcium soap and inorganic calcium salts occurs if the immersion solution contains ethylene diamine tetra-acetic acid. Compared with results for the previous year there was a marked improvement in the bacteriological state of liners as a result of the absence of calcium deposits. It has also been shown that the addition of tetramethylthiuram disulphide to the rubber mix

from which liners are made gives useful bactericidal properties. The intensity of the bactericidal action was little reduced after liners had been in farm use for 4 months.

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629. ANTIBIOTIC TREATMENT OF MASTITIS AND ITS EFFECT ON THE CELL CONTENT OF THE MILK

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Bacteriological examination and differential cell counts have been done on milk samples taken from a dairy herd over a period of 3 years, and attempts have been made to eliminate, by means of intramammary antibiotic treatment, streptococcal and staphylococcal infections. The present paper discusses the effect of eliminating the organisms from the milk on the cell counts of the milk.

METHODS

For the first half of the period samples of fore-milk were taken from individual quarters at weekly intervals and for the second half at fortnightly intervals. Differential cell counts were done by the method described by Blackburn & Macadam (1) and Blackburn, Laing & Malcolm (2), except that the milk smears were stained by Leishman's stain and not by the sudan black-Leishman method. It was found that the cells could be easily differentiated in smears of fresh formolized milk stained by Leishman's stain.

Bacteriological examination was done by smearing 0·01 ml. of milk on horse-blood agar within half an hour of taking the sample. Plates were read at 18 and 42 hr. Staphylococci were tested for coagulase; streptococci were typed only as to whether they were group B, group C or *Streptococcus uberis*.

The antibiotics used in intramammary treatment were penicillin, 100,000 units; a combination of penicillin, 100,000 units and dihydrostreptomycin, 0·1 g., or dihydrostreptomycin, 0·25 g. Courses of treatment varied from one tube on each of two consecutive nights to two tubes with a 3-day interval between them, or one tube on each of three consecutive nights.

All quarters treated were infected with coagulase-positive staphylococci or haemolytic streptococci, and the cell count of the milk of the quarters that were treated was always over 200,000/ml. and the percentage of cells present as polymorphs was high (65–95%).

If coagulase-positive staphylococci or haemolytic streptococci were found in the milk 1 or 2 weeks after treatment of an infected quarter, it was assumed that treatment had been unsuccessful. If, however, these organisms were not found till the third or fourth week after treatment, it was assumed that the quarters had become reinfected.

A quarter infected for 3 weeks or longer before treatment was taken as a long-standing infection, whereas if it was only infected for 2 weeks or less before treatment it was taken as a recent infection.

RESULTS

The number of cases of udder infection treated is shown in Table 1. Treatment eliminated staphylococci in 61% of infections and streptococci in 80% of infections. Owing to the use of more than one antibiotic and the different methods of treatment, too much

significance should not be attached to these figures, but it may be noted that organisms were eliminated in a higher percentage of recent infections than in those of long standing, both for staphylococci and streptococci.

Table 1. *Number of quarters treated and the number from which mastitis organisms were eliminated*

	No. of quarters treated	No. of quarters from which organisms were not cleared	No. of quarters from which organisms were cleared
Staphylococci:			
Recent infections	95	28 (30%)	67 (70%)
Long-standing infections	150	67 (45%)	83 (55%)
Total	245	95 (39%)	150 (61%)
Streptococci:			
Recent infections	42	6 (14%)	36 (86%)
Long-standing infections	51	13 (25%)	38 (75%)
Total	93	19 (20%)	74 (80%)

A return to normal cell count has been taken as a fall to 70,000 cells/ml. or less. Campbell (3) and MacLeod & Anderson (4) have reported this as a typical figure for the total cell content of milk from normal quarters. It was found that occasionally the cell count dropped to normal by the end of 1 week after treatment, but as a rule, the period was longer, even as long as 5 weeks. However, in all cases in which the cell count took the longer period to come down to normal, there was a marked change in the differential cell count at the end of the week after treatment. This change took the form of a drop in the percentage of polymorphs present in the total cells of the milk; a drop from over 65% to less than 30%.

Some quarters became reinfected before there was time for the cell count to drop to normal, but if the percentage of polymorphs dropped to below 30%, and at the same time or subsequently the total cell count fell to less than half of the pretreatment figure it was assumed that the cell counts would have fallen to normal if reinfection had not occurred.

In other quarters the cell count never dropped as low as 70,000 cells/ml. but took on the type of cell count seen in late lactation (5) with a polymorph percentage of less than 30%, the majority of the cells being cast epithelial cells. In a few such quarters the level of the cell count matched that of the other quarters of the udder if the cow was in late lactation. In the majority, however, the figure remained higher than that of the other quarters, and it appeared as if the quarter in question were drying off prematurely.

In some quarters the cell counts remained similar to those obtaining before elimination of the organisms.

Table 2 summarizes the results of the elimination of staphylococci and streptococci from 224 quarters. In 188 quarters (84%) the cell counts were reduced. If staphylococcal and streptococcal infections are taken separately the percentage in which the cell counts were reduced is of the same order, 83 and 87% respectively. If, however, the figures for recent and long-standing infections are examined, it is seen that with streptococcal infections a lower percentage of long-standing infections showed a drop in cell count than recent infections, 74 and 100% respectively, whereas in staphylococcal infections the reverse was the case, 86 and 79%.

In thirty-six quarters (16%) the cell count remained unchanged after removal of the organisms, and twenty-nine of these quarters have been examined through subsequent lactations. Nineteen of the twenty-nine quarters started the next lactation with normal cell counts, eight had high cell counts associated with staphylococci and two had high cell counts with no organisms.

Table 2. *Cell counts after eliminating mastitis organisms by treatment*

	No. of quarters cleared of organisms	No. of quarters in which cell count was unchanged	No. of quarters in which cell count was reduced
Staphylococci:			
Recent infections	67	14 (21%)	53 (79%)
Long-standing infections	83	12 (14%)	71 (86%)
Total	150	26 (17%)	124 (83%)
Streptococci:			
Recent infections	36	0 (0%)	36 (100%)
Long-standing infections	38	10 (26%)	28 (74%)
Total	74	10 (13%)	64 (87%)
Combined:			
Recent infections	103	14 (14%)	89 (86%)
Long-standing infections	121	22 (18%)	99 (82%)
Total	224	36 (16%)	188 (84%)

Table 3. *The numbers of quarters in which the cell counts were reduced after elimination of mastitis organisms and the incidence of reinfection*

		No. of quarters in which cell count was reduced		
	Total	Reduced to normal	Reduced but quarter became reinfected	Reduced to late lactation count
Staphylococci:				
Recent infections	53	31 (59%)	9 (17%)	13 (24%)
Long-standing infections	71	25 (35%)	20 (28%)	26 (37%)
Total	124	56 (45%)	29 (23%)	39 (32%)
Streptococci:				
Recent infections	36	25 (70%)	3 (8%)	8 (22%)
Long-standing infections	28	9 (32%)	5 (18%)	14 (50%)
Total	64	34 (53%)	8 (13%)	22 (34%)
Combined:				
Recent infections	89	56 (63%)	12 (13%)	21 (24%)
Long-standing infections	99	34 (34%)	25 (25%)	40 (41%)
Total	188	90 (48%)	37 (20%)	61 (32%)

The extent to which the cell counts were reduced is summarized in Table 3. Of the 188 quarters in which the cell count was reduced after elimination of organisms 48% were reduced to normal; 20% were reduced, but the quarter became reinfected before there was time for the cell counts to come down to normal, and 32% were reduced to late lactation cell counts.

If the figures for recent and long-standing infections are compared it is seen that the percentage of recent infections returning to normal cell counts was almost twice that of long-standing infections (63 to 34%).

The percentages for staphylococcal and streptococcal infections taken separately are similar to those for the combined infections, except that the percentage of streptococcal infections that became reinfected during the first five weeks after treatment was only about half that for staphylococcal infections (13 and 23% respectively).

DISCUSSION

If the differential cell count is used to check whether treatment of mastitis has been successful it is evident that a period of at least 5 weeks after treatment must be allowed for the cell count to return to normal. Earlier examination, however, will indicate by a decrease in the percentage of polymorphs present in the milk whether the cell counts are likely to be reduced. In the present work the cell counts were reduced in 84% of quarters from which organisms were eliminated. A high proportion of the quarters in which the cell counts were not reduced resumed normal cell counts in the subsequent lactation. This suggests that whatever was causing the high cell counts disappeared during the dry period. It should be noted that quarters showing a high cell count with a high percentage of polymorphs, after elimination of the organisms, as a rule, showed a reduced milk yield in comparison to the other quarters of the udder. They may therefore have suffered trauma from the milking machine since they would be milked out more quickly than the other quarters. This could cause traumatic mastitis.

Though the cell count was reduced in 188 quarters, in only about half of them did it return to normal. In the remainder the cell counts became similar to those of late lactation milk in which the majority of the cells were epithelial cells or the quarters became reinfected. As a rule the quarters showing the late lactation type of cell count assumed normal cell counts in the subsequent lactation. That the length of time a quarter is allowed to remain infected with mastitis organisms has some bearing on the chance of that quarter to return to a normal cell count in the current lactation is borne out by the fact that only 34% of quarters with long-standing infections returned to normal cell counts compared with 63% for recent infections.

SUMMARY

Antibiotic treatment eliminated organisms from 61% of staphylococcal and from 80% of streptococcal udder infections. In 84% of quarters from which organisms were eliminated the cell counts of the milk were reduced, about half of them to a normal and about one-third to a late-lactation type of cell count in which the majority of the cells are epithelial cells. The remainder became reinfected before there was time for the cell counts to return to normal. It was found that it may take as long as 5 weeks for the cell counts to return to normal after elimination of mastitis organisms, but in quarters in which the cell count eventually fell there was a decrease in the percentage of polymorphs in the milk to less than 30% one week after treatment.

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630. THE DETERMINATION OF LACTOSE IN MILK

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INTRODUCTION

A considerable number of chemical and physical methods for the determination of lactose in milk are now available. With the exception of the direct volumetric method of Lane & Eynon (7) the determination is carried out on a protein-free serum prepared by precipitating the proteins with one of a variety of clearing agents. In spite of the fact that different clearing agents are known to give different values for the concentration of lactose in the prepared serum little work has been done to determine which coagulating agent produces a serum containing all of the lactose in a condition in which it can be determined accurately by one of the many chemical or physical methods available. McDowell (8) examined a number of methods in general use, and, on the assumption that those methods which gave results in close agreement with the direct volumetric method of Lane & Eynon were most likely to be correct, he finally concluded that the most suitable clearing agents for the polarimetric or iodiometric determinations of lactose were zinc and cadmium hydroxides. Clarification with strongly acid agents such as acid mercuric nitrate or phosphotungstic acid was found to give high values for lactose compared with the direct volumetric method, and McDowell concluded from this that these strongly acid reagents failed to remove substances which interfered with the subsequent determination of lactose. Gorbach & Schafer (4) found that when milk was cleared with zinc ferrocyanide some lactose was absorbed by the protein coagulum, and they suggested that the amount so absorbed compensated for the error introduced by neglecting the volume of the protein precipitate. Francis & Smith (2) showed that the results for lactose determined on sera prepared by coagulating milk with various clearing agents showed a variation up to 10% according to the agent used. They found that dialysed iron gave the highest value for lactose and recommended the use of this reagent without, however, giving satisfactory evidence as to why this reagent should be used in preference to any other. Block, Bolling, Weiss & Zweig (1) found that only 87% of whey protein was precipitated by zinc hydroxide at pH 8·9 and that of a number of metallic salts and hydroxides tested, only $FeCl_3$ at pH 4·3 gave complete precipitation of the protein from whey. They found that dialysed iron removed only 50% of the protein from whey.

The clarifying agents normally used to prepare protein-free sera for the determination of lactose are either metallic salts or their hydroxides or complex acids such as phosphotungstic acid. Coagulation is carried out usually without heating over a wide pH range. If the serum is clear it is usually considered satisfactory for the determination of lactose.

Errors in the determination of lactose may arise during the preparation of the protein-free serum through (1) the incomplete precipitation of protein, (2) the absorption of lactose by the coagulated protein, and (3) extensive hydration of the protein coagulum which results in a concentration of lactose in the serum. While a correction factor can be,

and is, applied to compensate for the volume of the protein precipitate, it should be noted that varying amounts of adsorbed water are retained by the protein coagula prepared with different clearing agents and that the protein correction factor must necessarily vary with the clearing agent used.

The precise mechanism by which proteins are precipitated from solution by metallic salts and complex acids is still not fully understood. The conditions governing the combination of protein and metal ions have been investigated by Klotz(6), and the fact that some metal-protein complexes remain soluble while others are insoluble appears to be due to the varying extent to which the protein loses its lyophilic properties through dehydration when water molecules are eliminated from charged centres within the protein molecule by the preferential attraction of these centres for metal ions. Where the metal ions compete successfully against water dipoles for the charged centres the elimination of adsorbed water molecules is fairly complete and the precipitation of the metal-protein complex results.

According to Klotz, metal-protein complexes are formed by the electrostatic attraction of the positively charged metal ions for electron-donating groups situated in or on the protein molecule. If we exclude certain specific reactions which might be expected to occur between metals such as copper and active groups such as the sulphydryl group, the electron-donating groups of protein which are of importance in binding metal ions are the available side-chain carboxyl groups of di-carboxylic acids, similarly placed amino groups of diamino-acids and the free carboxyl and amino groups at the ends of the polypeptide chains. An important point to note is that the carboxyl groups are in a position to donate electrons only when they are ionized while amino groups can do so only when unionized. The strength of binding of metal ions such as Zn^{2+}, Cd^{2+} and Cu^{2+} by acid proteins such as casein is therefore greatest at pH values above the isoelectric points of the proteins, since both carboxyl and amino groups are then in a position to donate electrons; the metal-binding power of the protein can be expected to increase progressively with rise in pH value to a point where it fails to meet the increasing competition of hydroxyl ions for the available metal ions. In acid solutions the strength of binding of metal and protein depends on the extent to which the metal ion can compete successfully against hydrogen ions for the complexing groups of protein. Little binding of Zn^{2+}, Cd^{2+} or Cu^{2+} occurs in strongly acid solutions and as protein coagulants in acid solutions these metals are of little value. On the other hand Hg^{2+} is able to compete successfully against hydrogen ions even in strongly acid solutions, mercuric nitrate being effective as a clearing agent at pH 1·0.

At pH values below the isoelectric points of proteins the decreased ionization of carboxyl groups and the increased ionization of amino groups results in a reversal of charge on the protein. These groups now act as electron acceptors and possess an affinity for negatively charged ions. Phosphotungstic acid therefore forms stable complexes with proteins at pH values below their isoelectric points in the same way as stable complexes are formed between metal ions and protein at pH values above them, the complexes being generally sufficiently insoluble for the complete precipitation of protein to be effected.

With milk or other protein solution containing aldose sugar a complication arises in that a Malliard type of reaction can occur involving the aldehyde group of the sugar and the free amino groups of protein. The amino groups concerned in this reaction are the

free unionized groups present either as side chains of diamino-acids or terminal α groups of other amino-acids. The reaction proceeds only when the groups are unionized, i.e. at pH values above the isoelectric points of proteins. Although the loss of amino groups through the formation of sugar-protein complexes results in the loss of charged centres on the protein molecule at which hydration can occur, this is more than balanced by the increase in lyophilic properties induced by the presence of carbohydrate in the complex. The powerfully lyophilic nature of these complexes has been demonstrated by Mohammed, Fraenkel-Conrat & Olcott (9), who showed that the combination of heat-labile blood albumin with glucose produced a complex which remained uncoagulated after heating at 100° C. for several hours. The elimination of amino groups by sugar-protein interaction also results in a reduced affinity of the protein for metal ions, and as the latter are not held so strongly by the sugar-protein complex as by the intact protein they may be unable to effect the precipitation of the protein from the complexes. An important point to note is that the sugar in these complexes is bound by its aldehyde group and it therefore displays neither reducing properties nor its full optical activity; low values for the sugar content of sera containing these complexes would be recorded whatever the method employed for its determination. The direct determination of lactose in unclarified milk by oxidation with Fehling's solution does not overcome this difficulty since at the high pH value and high temperature operating during the oxidation the formation of sugar-protein complexes is encouraged.

There is now a considerable accumulation of evidence that lactose and protein combine to form complexes of varying stability in heated milk. That lactose and protein associate to form complexes in unheated milk can be expected on purely theoretical grounds and on the basis of infra-red spectra measurements Goulden (5) has produced evidence that such associations do, in fact, occur. The stability of these complexes towards clearing agents is obviously of importance in the quantitative examination of milk for lactose. Ideally the clearing agent should break up lactose-protein complexes with the complete regeneration of the lactose while simultaneously removing the whole of the protein from solution. Further, the coagulation should be carried out at its isoelectric point to reduce the degree of hydration of the protein coagulum to a minimum.

On the basis of these considerations it would appear that the conclusions drawn by various workers regarding the most suitable clarification agent for the determination of lactose in milk are in doubt. Low values recorded for sera prepared by precipitating the milk proteins with zinc or cadmium hydroxides might possibly be due to the formation of lactose-protein complexes which are left intact after interaction of the protein with metal ions at high pH values. The higher values given by sera prepared with strongly acid agents such as acid mercuric nitrate and phosphotungstic acid may be due to the liberation of lactose from lactose-protein complexes at the low pH value employed, but they may be due also to an increase in the amount of bound water in the protein coagulum resulting in an increase in the lactose content of the serum. The following experiments were carried out to determine (*a*) the extent to which the pH, at which clearing agents operate, influences the lactose content of the serum either through the effects on lactose-protein interaction or the degree of hydration of the coagulum, and (*b*) the most suitable clearing agent which would reduce to a minimum any errors introduced through these two factors.

EXPERIMENTAL

Reagents

Zinc acetate. 30 g. crystals ($Zn(OOC.CH_3)_2.2H_2O$) dissolved in water and the solution diluted to 100 ml.

Sodium hydroxide. 10 g. dissolved in water and the solution diluted to 100 ml.

Potassium ferrocyanide. 15 g. dissolved in water and the solution diluted to 100 ml.

Phosphotungstic acid in HCl. 50 g. sodium tungstate crystals ($Na_2WO_4.2H_2O$) and 2·4 g. disodium phosphate (anhydrous) were dissolved in warm water, 44 ml. conc. HCl added and the solution diluted to 250 ml. This reagent must be stored in a warm place to prevent crystallization.

Phosphotungstic acid in acetic acid. 20 g. phosphotungstic acid were dissolved in water, 20 ml. glacial acetic acid added and the solution diluted to 100 ml.

Acid mercuric nitrate. A.O.A.C. reagent (11). Mercury was dissolved in twice its weight of conc. HNO_3 and the solution diluted with an equal volume of water.

Oxidized acid mercuric nitrate reagent. The reagent prepared as above contained considerable quantities of nitrite which was removed by heating the reagent to 60° C. and oxidizing the nitrite with solid potassium permanganate until a permanent precipitate of manganese dioxide formed. The reagent was then filtered.

Zinc acetate-phosphotungstic acid reagent. 25 g. zinc acetate and 12·5 g. phosphotungstic acid were dissolved in water, 20 ml. glacial acetic acid added and the solution diluted to 100 ml.

Materials and procedures

Separated milk was used throughout. The milk was obtained from the Milk Marketing Board's depot at Newbury and separated in the University's Department of Dairying.

Measurements of optical rotatory power were made in 2 dm. tubes in a Hilger Polarimeter, Model no. M413, using a sodium lamp as illuminant.

The volume of coagulated protein was determined by the method of Scheibler (10). The results obtained by this method are dependent on very accurate determinations of optical rotatory power of diluted milk sera. The results given in the tables are average values of several determinations.

RESULTS AND DISCUSSION

Zinc acetate with varying amounts of NaOH or HCl were mixed in the presence of milk to produce protein coagula at pH values varying from 4·6 to 7·4. The results given in Table 1 show that with a fall in pH there is a progressive increase in serum N, indicating that zinc ions are unable to compete successfully with hydrogen ions for the complexing groups of protein and that the precipitation of protein by Zn^{2+} is only complete at pH values above 6·8. With a fall in pH value from 7·4 there is a slight rise in rotation of the serum to a value which remains practically constant from pH 7·4 to 6·4. The rotation falls rapidly when the pH changes from 6·4 to 5·8 and then it rises to a low, almost constant, value which persists over the pH range 5·0–4·6. When the sera prepared as above were further treated with a solution of phosphotungstic acid in acetic acid to give sera at a pH value of 4·6 the serum N was reduced to a low level which was practically the same for all sera and approached closely the lowest value recorded for zinc hydroxide

alone while, at the same time, the rotation increased to a maximum constant value (see Table 2). This value was only slightly higher than that given by a serum prepared with *phosphotungstic acid in HCl (see* Table 1). These results show that zinc hydroxide sera contain lactose in a form in which it cannot be determined polarimetrically and that phosphotungstic acid regenerates this lactose to a form in which it displays its full optical activity. The fact that this regeneration is effected by a reagent reacting strongly with

Table 1. *The influence of* pH *on the optical rotation and residual nitrogen content of zinc hydroxide sera*

(80 ml. milk was used in each test and the mixture of milk and clearing agent diluted to 100 ml.)

Test no.	Coagulant	Alkali or acid added	pH	Rotation	N in serum (%)
1	8 ml. Zn acetate	8 ml. 10% NaOH	7·4	4·44	0·045
2	8 ml. Zn acetate	6 ml. 10% NaOH	7·2	4·47	0·027
3	8 ml. Zn acetate	4 ml. 10% NaOH	6·8	4·47	0·029
4	8 ml. Zn acetate	2 ml. 10% NaOH	6·4	4·46	0·049
5	8 ml. Zn acetate	0 ml. 10% NaOH	5·8	4·15	0·098
6	8 ml. Zn acetate	2 ml. 2N-HCl	5·4	4·21	0·098
7	8 ml. Zn acetate	4 ml. 2N-HCl	5·0	4·23	0·096
8	8 ml. Zn acetate	6 ml. 2N-HCl	4·8	4·24	0·097
9	8 ml. Zn acetate	8 ml. 2N-HCl	4·6	4·24	0·100
10	20 ml PTA in HCl	—	1·8	4·60	0·030

PTA = phosphotungstic acid.

Table 2. *The change in optical rotation and nitrogen content of zinc hydroxide sera prepared at different* pH *values when further treated with phosphotungstic acid in acetic acid*

(In each test 40 ml. $Zn(OH)_2$ serum and 5 ml. phosphotungstic acid in acetic acid were used and the mixture diluted to 50 ml.)

Test no.	Rotation		N in serum (%)
	Observed	Corrected*	
1	3·64	4·55	0·028
2	3·65	4·56	0·028
3	3·66	4·57	0·028
4	3·69	4·61	0·031
5	3·69	4·61	0·032
6	3·69	4·61	0·031
7	3·70	4·62	0·031
8	3·70	4·62	0·032
9	3·70	4·62	0·032

* The observed rotations were multiplied by 5/4 to make them comparable with those in Table 1.

unionized amino groups suggests that the inactive lactose in the zinc hydroxide serum is, in fact, present as a lactose-protein complex. If lactose is present in this form it should be possible to free it from such complexes or prevent their formation by pre-treating milk with formalin before coagulating the milk proteins with zinc hydroxide. Table 3 records the results of such pre-treatment and gives also the effect of formalin on other coagulating agents in common use.

The results show that increasing additions of formalin appreciably increase the rotation of a zinc hydroxide serum but exert little effect on sera cleared either with acid mercuric nitrate or phosphotungstic acid. The maximum value given by formalin-treated zinc hydroxide sera approaches closely the values given by the more acid reagents without added formalin. These results indicate that lactose associated with the amino groups of protein in milk is unable to compete with added formalin for these groups and is therefore

released to the serum when the milk proteins are precipitated with zinc hydroxide. Further, as formalin has little effect on the rotations of mercuric nitrate or phosphotungstic acid sera, lactose-protein complexes are apparently absent from these sera. This is to be expected, as lactose and formalin can only react with the amino groups of *protein when the latter are unionized. At the low pH values of mercuric nitrate and* phosphotungstic acid sera it can be assumed that the free amino groups of protein are strongly ionized and therefore not in a position to fix either lactose or formalin.

Table 3. *The influence of added formalin on the optical rotations of milk sera cleared with different clearing agents*

(40 ml. milk was used in each test and the mixture of milk and clearing agent diluted to 50 ml.)

Test no.	Coagulant	40% formalin added (ml.)	Rotation
1	4 ml. Zn acet. + 2 ml. 10% NaOH	None	4·29
2	4 ml. Zn acet. + 2 ml. 10% NaOH	1	4·40
3	4 ml. Zn acet. + 2 ml. 10% NaOH	2	4·47
4	2 ml. acid $Hg(NO_3)_2$	None	4·46
5	2 ml. acid $Hg(NO_3)_2$	2	4·47
6	8 ml. PTA in HCl	None	4·47
7	8 ml. PTA in HCl	2	4·49

Taking the results shown in Tables 1–3 together it is clear that zinc hydroxide, because it operates at a high pH value, is not a satisfactory clearing agent for the determination of lactose in milk. Acid mercuric nitrate and phosphotungstic acid give values which closely approach one another with phosphotungstic acid giving the slightly higher rotation. Acid mercuric nitrate as a clearing agent for milk has been criticized by Garrison (3) on account of the slowness of its action. He recommended fortifying the reagent with phosphotungstic acid. It can be further criticized in that the reagent when prepared according to the directions given by A.O.A.C. (11) contains appreciable quantities of nitrite, and this reacts with free amino groups of protein to produce gaseous nitrogen. The gas appears in appreciable amounts in polarimeter tubes which have been allowed to stand for some time. These effects due to nitrite can be removed by oxidizing the fresh reagent with solid permanganate.

The higher rotations given by acid mercuric nitrate and phosphotungstic acid sera are due in part to the greater release of lactose to the sera as indicated above, but as these reagents operate at very low pH values there is the possibility that appreciable amounts of adsorbed water are attracted to the highly ionized amino groups of protein resulting in a concentration of lactose in the serum. This hydration effect should be reduced to a minimum by coagulating the milk proteins at their isoelectric points. As equal numbers of amino and carboxyl groups are ionized at this pH value the most suitable coagulant must necessarily be one which reacts with both of these groups and for this reason the following reagent was prepared:

Zinc acetate-phosphotungstic acid reagent. Zinc acetate (25 g.) and phosphotungstic acid (12·5 g.) were dissolved in water, 20 ml. glacial acetic acid added and the mixture diluted to 100 ml. 5 ml. of this solution added to 40 ml. milk with dilution to 50 ml. produced a clear filtered serum with a pH value of 4·6.

Milk was treated with different clearing agents and the volume of the protein precipi-

tate determined by the method of Scheibler (10). Table 4 gives the results of these determinations along with the observed and corrected rotations of the sera.

The results show that there is quite considerable variation in the degree of hydration of the protein coagula produced by different clearing agents. Phosphotungstic acid operating at a very low pH value now appears unsatisfactory in that the volume of the protein precipitate is raised to a particularly high value through extensive hydration, and associated with this high value is a correspondingly high value for the rotation of the serum. With acid mercuric nitrate values for the volume of the coagulum varied widely;

Table 4. *The volume of the protein precipitate produced by different clearing agents and its effect on the rotation of the serum*

(The volume of milk used in each test was 40 ml.)

Test no.	Coagulant	Vol. of protein ppt. (ml./100 ml. milk)	Rotation	
			Observed	Corrected*
1	4 ml. Zn acet. + 2 ml. 10% NaOH	3·9	4·39	4·22
2	4 ml. Zn acet. + 4 ml. $K_4Fe(CN)_6$	4·4	4·48	4·29
3	10 ml. PTA in HCl	5·2	4·50	4·27
4	2 ml. acid $Hg(NO_3)_2$	—	4·48	—
5	3 ml. oxidized $Hg(NO_3)_2$	3·5	4·49	4·34
6	5 ml. Zn acet. + PTA	3·1	4·49	4·35

* Corrected for the volume of the precipitate.

Table 5. *The influence of* pH *on the clearing action of the zinc acetate-phosphotungstic acid reagent*

Test no.	pH	Vol of protein ppt. (ml./100 ml. milk)	Rotation		N in serum (%)
			Observed	Corrected	
1	5·9	4·4	4·38	4·28	0·025
2	5·1	3·5	4·53	4·37	0·028
3	4·8	3·3	4·54	4·39	0·028
4	4·6	2·9	4·55	4·42	0·028
5	4·4	4·1	4·56	4·37	0·028
6	4·1	5·6	4·56	4·31	0·028
7	3·8	5·9	4·54	4·28	0·027

they were always much smaller than was theoretically possible and were even occasionally negative. There appears to be some factor in the use of this reagent which increases the optical rotatory power of the constituents of the serum on dilution with water. Since the oxidized reagent does not show the same peculiarities the interfering factor is probably nitrite. The lowest value for the volume of the protein precipitate was given by the zinc acetate-phosphotungstic acid reagent, indicating that this reagent was the most effective in eliminating adsorbed water from the coagulum. Associated with this low volume of coagulum was the highest corrected rotation of the serum which further indicates that this reagent was the most effective in releasing bound lactose to the serum. Of the reagents tested therefore the zinc acetate-phosphotungstic acid reagent produced the most satisfactory serum for the determination of lactose. Since the isoelectric points of the milk proteins extend over the pH range 4·1–5·2, experiments were conducted with the zinc acetate-phosphotungstic acid reagent to determine the pH value at which coagulation of the proteins most closely approached completion and the degree of hydration of the protein precipitate was a minimum. The results are given in Table 5.

They show that the reagent produces a serum essentially free from protein over a wide pH range, but that hydration of the coagulum increases significantly if the pH rises or falls appreciably from pH 4·6. When the reagent is prepared as described above, i.e. containing acetic acid and used in the proportion of 5 ml. reagent to 40 ml. milk, the pH of the serum is, in fact, 4·6.

The average volume of coagulated protein from 100 ml. milk using this reagent at pH 4·6 was 2·9 ml., which corresponded to a protein content of 3·45% in the milk. This gives a value of 0·84 for the specific volume of the precipitated proteins. The generally accepted value for the specific volume of dry-milk protein is 0·743, so that the coagulum obtained by the zinc acetate-phosphotungstic acid reagent is slightly hydrated.

When clearing agents are added to whole milk the fat is entrained by the protein coagulum increasing its volume, so that a correction factor for the fat content of the milk must be applied. The evaluation of this correction factor is based on the average specific gravity of milk fat which is 0·93; this gives a value of 1·07 for the specific volume of milk fat.

Details of suggested procedure

To 40 ml. milk in a 50 ml. graduated flask add 5 ml. zinc acetate-phosphotungstic acid reagent, dilute to the mark without mixing (to avoid the formation of a foam) and then mix by *vigorous* shaking. Filter through a dry filter into a dry flask and polarize the filtrate in a 2 dm. tube in a polarimeter illuminated with sodium light. Correct the observed reading for the volume of protein and fat as follows:

$$\text{'corrected reading} = \text{observed reading} \times \frac{100 - 0{\cdot}8\,(P \times 0{\cdot}84 + F \times 1{\cdot}07)}{100}$$

where P and F are the percentages of protein and fat in the milk. The specific rotation (α) of lactose monohydrate at 20° C. using sodium light is given by the expression

$$[\alpha]_D^{20} = \frac{100a}{l \times c} = 52{\cdot}5,$$

where a is the observed rotation, l the length of the tube in decimetres and c is the concentration of lactose hydrate in g./100 ml. solution. The number of g. lactose hydrate in 100 ml. prepared serum (c_s) is therefore given by

$$c_s = \frac{100 \times r}{52{\cdot}5 \times l},$$

where r is the rotation corrected for the volume of fat and protein. The percentage of lactose hydrate in the milk (c_m) in g./100 ml. is

$$c_m = \frac{100 \times r}{52{\cdot}5 \times l} \times \frac{100}{80} \quad \text{which simplifies to} \quad \frac{5 \times r}{4{\cdot}2},$$

if a 2 dm. tube is used.

SUMMARY

One of the most important stages governing the accuracy of lactose determinations in milk is the preparation of the protein-free serum. Factors influencing the lactose content of the serum are: (1) the complete precipitation of protein, (2) the formation of lactose-protein complexes, and (3) the degree of hydration of the protein coagulum.

Experiments are described which show how these factors are influenced by different clearing agents operating at different pH values. On the basis of the results obtained a reagent containing zinc acetate, phosphotungstic acid and acetic acid is suggested; it reduces to a minimum any errors introduced by the above factors.

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637.127.17

631. VARIATIONS IN IODINE VALUE OF MILK FATS*

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(With 6 Figures)

The iodine value of a fat is determined by its content of unsaturated fatty acids. In many fats the proportions of the constituent acids are relatively constant and their iodine values within narrow limits characteristic of them. In such cases the estimation of iodine value provides a rapid and simple means of establishing the identity and purity of a fat sample. This is not so with cow milk fat, however, since it varies widely in its iodine value owing to the unusually wide range of fatty acids constituting its triglycerides.

In cow milk fat saturated acids of chain lengths 4–20 carbon atoms (i.e. even-numbered) predominate (Hilditch and co-workers (8); Hansen & Shorland (7)), but acids with uneven chain lengths (Shorland (19)) as well as with branched chains have also been reported (Hansen & Shorland (6)). The unsaturated acids occur in chain lengths of 10–20 carbons, but the principal unsaturated acid, as in most other animal fats, is oleic. Short-chain monoethenoid acids of molecular weight lower than C_{18} and C_{16}, amounting to about 5% by weight of the unsaturated acids, also occur, but in many analyses they are not distinguished from oleic acid and are included with it. Moreover, di- and tri-ethenoid C_{18} acids, amounting to about 5–8% of the unsaturated fraction, have recently been found to be considerably more complex than was formerly believed (Shorland (18)).

Many factors influence the chemical composition of cow milk fat, and a number of investigations have been made to elucidate their relative importance. (References to the extensive literature on this subject will be found in the books of Hilditch (8) and Espe (4)). The main factors are environmental and nutritional in nature and they cannot always be differentiated.

Experiments with lactating cows have been made under a wide range of conditions, some of them remote from practical farming. In many experiments the animals have been stall-fed without access to pasture. Besides the effects of different feedstuffs, Smith & Dastur (21) showed that the composition of milk fat was markedly affected by inanition, which caused a decrease in the lower acids and a marked increase in the oleic acid content.

It is the purpose of this paper to discuss causes of variation, as shown by difference in iodine value, in the composition of milk fat of cows maintained under all-the-year-round grazing conditions typical of dairy farming in the North Island of New Zealand. Recent research work with 'labelled' metabolites has emphasized the importance of short-chain fatty acids derived from rumen fermentation of carbohydrates as precursors of the lower acids (C_4 to C_{14}) of milk fat (Popjak (14)). On a weight percentage basis,

* This paper includes results from a thesis presented by J. W. Mayhead in partial fulfilment of the requirements of the degree of M.Agr.Sc.

however, milk fat consists chiefly of long-chain acids (C_{16} to C_{18}), and results presented in this paper support the theory that the long-chain unsaturated acids in herbage lipids contribute materially to the milk fat secreted by dairy cows.

EXPERIMENTAL

Most of the experiments required estimations of the iodine value of milk fats from several dairy cows maintained under prescribed conditions (usually normal grazing) for considerable periods. In some experiments cows were fed with an unsaturated vegetable oil, and the incorporation of ingested fatty acids in the milk fat was followed by means of its increased iodine value. Peanut (ground nut) oil was used because (*a*) its unsaturated fraction consists almost entirely of oleic and linoleic acids, in which it resembles qualitatively cow depot and milk fats, and (*b*) when fed to dairy cows it appears to have little effect on the milk fat except to raise its oleic acid content (Hilditch (8)). The amounts of peanut oil fed, 8–16 oz. day, were of the order of the amounts of triglyceride normally ingested in herbage by grazing dairy cows (see also p. 246).

Animals and feeding. The animals were mature dairy cows, those used in the experiments described under paragraphs 6 and 7 below being in the later stage of lactation. They were maintained at liberal standards of grazing and in some cases an allowance of meal was made. (The oil in the meal amounting to about $1\frac{1}{2}$ oz. per day was common to both the experimental and control groups.) The peanut oil (iodine value 96·2) was either (*a*) mixed with the meal or (*b*) given as a drench. When given as a drench, precautions were taken to ensure that the viscous nature of the oil did not interfere with the accuracy of the dosing. Milkings were at 5 a.m. and 4 p.m.

Preparation and analysis of milk fats. Fat yields were estimated from milk weights and fat tests (Gerber). Butters were churned in the laboratory from representative samples of cream obtained by overnight settling of the milk. Milk fats prepared from butters by melting and filtering at low temperatures were stored in a refrigerator pending analysis.

Iodine values were estimated by Wijs' method using approximately 100% excess reagent and allowing the reaction to proceed for 30 min. in the dark. Results of duplicate determinations agreed within 0·2 units or less.

RESULTS

(1) *Differences between Friesian and Jersey cow milk fats.* Five cows in each group were selected for closeness of calving dates. Samples of milk fat from combined morning and evening milkings were obtained on eleven occasions during the period October (i.e. spring, about 2 months after calving) to January 14 months later. Results are shown in Table 1.

On the average the iodine values of Jersey milk fats were lower by 2·2 units than those of the Friesians.

(2) *Seasonal variations.* Both series showed the same trend, being lowest in summer (December) and highest in winter. The maximum differences were 5·4 (Jerseys) and 7·6 (Friesians), average 6·5 units, and are of the same order as those found by Cox & McDowall (2) for fats from New Zealand commercial butters.

(3) *Individual variations.* It is apparent from Figs. 1 and 2 that the milk fats from six cows at similar stages of lactation and fed under liberal grazing conditions had iodine

values showing marked individual differences (nearly 5 units) which persisted throughout the trial and which were still in evidence even when the iodine value was raised by oil feeding.

Table 1. *Iodine values of milk fats of Friesian and Jersey cows (five cows in each group)*

Date of milking	Friesian	Jersey
31. x.	35·0	33·5
5. xii.	33·2	30·9
5. i.	36·4	35·3
7. ii.	35·7	33·7
7. iii.	36·9	35·8
7. v.	40·4	35·2
14. vi.	39·1*	—
Dry period	—	—
4. ix.	37·1	35·5
6. x.	36·2	34·2
10. xii.	32·8	30·4
7. i.	36·5	33·5
Average	36·0	33·8

* Excluded from average.

(4) Diurnal variations. In Figs. 1 and 2 it is seen that the iodine values of the morning samples were lower than the evening ones (on the average by 1·8 units), the maximum difference being 4·9 units. (The averaged results are shown in Fig. 3.)

(5) *Incidental variations.* Variation in iodine values of daily composite samples of milk fat (to which regular seasonal and diurnal variations undoubtedly contributed to some extent), were less than the regular diurnal variations, the maximum being 3·6 units, and they showed no regular trend (Fig. 4).

(6) *Feeding experiments.* The feeding experiments were carried out with cows grazing on pasture ample for lactation. In order to facilitate the feeding of oil, it was mixed with 1 lb. of meal (0·6 lb. S.E.) and the meal was also given to the control animals. During periods of partial inanition the experimental cows were given oil, hay and concentrates while the controls were given hay and concentrates only, the allowance for each group being about 5·5 lb. S.E. per day, i.e. an estimated "maintenance ration". One half of the ration was given after the morning milking and the other after the evening milking.

The rate and extent of transfer of ingested fatty acids was studied by means of the following experiments:

(*a*) Iodine values of morning and evening milk fats of six Jersey cows were followed for 16 days. Three of the cows were maintained as controls throughout the period, but from the third day the other three cows were each given 8 oz. peanut oil for seven consecutive mornings. Milks were sampled during this period and for a further 7 days. Results are shown in Figs. 1, 2 and 3. On the 5th, 6th and 7th days after oil feeding began the iodine values had attained a maximum difference, being, on the average, 4·4 units higher than the controls on the same days. (Analysis of variance of the results for the 7 day period during which the effects of oil ingestion were evident (2nd to 8th days after feeding it) indicates that the increases were highly significant.) During this trial the average yields of milk fat per day for the experimental and control groups were respectively 1·20 and 1·35 lb. and the milk yield was not affected by the oil feeding.

(*b*) In this experiment (Figs. 5 and 6) 16 oz. peanut oil per day were fed, one half at each milking. Three Friesian cows were used, two experimental animals and one control.

Yields of milk fat from oil-fed and control animals were respectively 0·90 and 0·70 lb., on the average per day. The increases in iodine value when the values were at a fairly steady level towards the end of the 16 day period were 9·8 (Fig. 5) and 8·9 (Fig. 6), average 9·4 units.

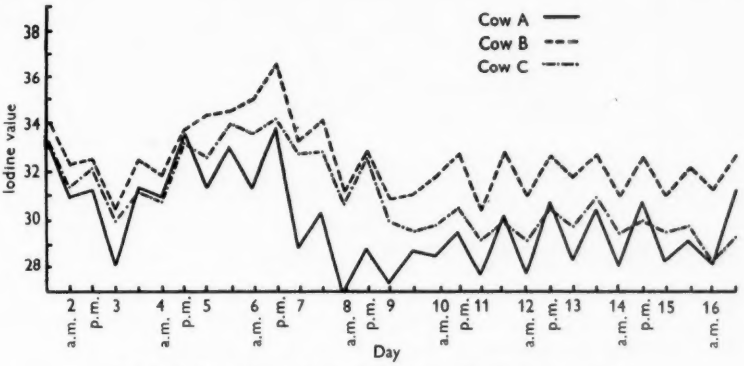


Fig. 1. Iodine values of milk fats of three Jersey cows (controls).

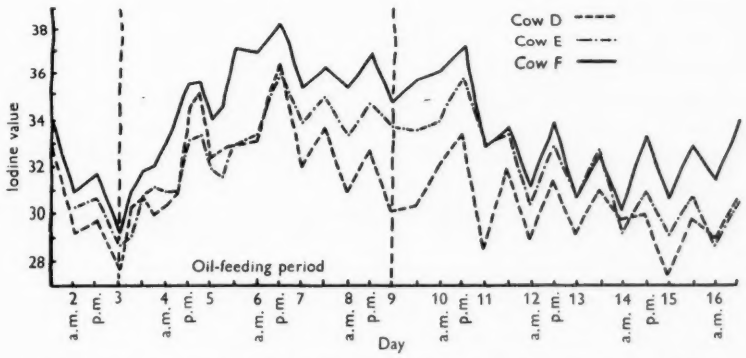


Fig. 2. Iodine values of milk fats of three Jersey cows fed 8 oz. peanut oil per day during 3rd–9th days.

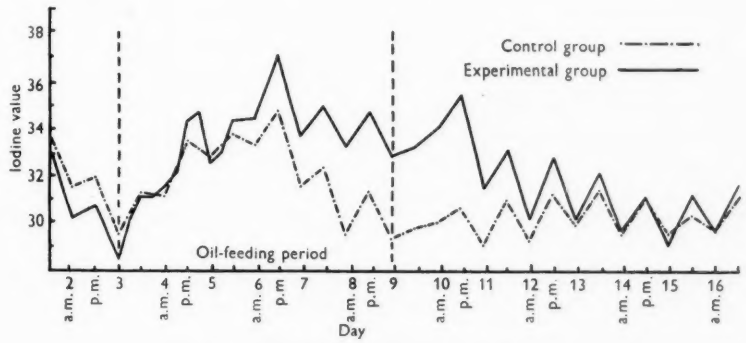

Control group
Experimental group
Iodine value
38
36
34
32
30
Oil-feeding period
2 a.m. p.m. 3 4 a.m. p.m. 5 6 a.m. p.m. 7 8 a.m. p.m. 9 10 a.m. p.m. 11 12 a.m. p.m. 13 14 a.m. p.m. 15 16 a.m.
Day

Fig. 3. Average iodine values from Figs. 1 and 2.

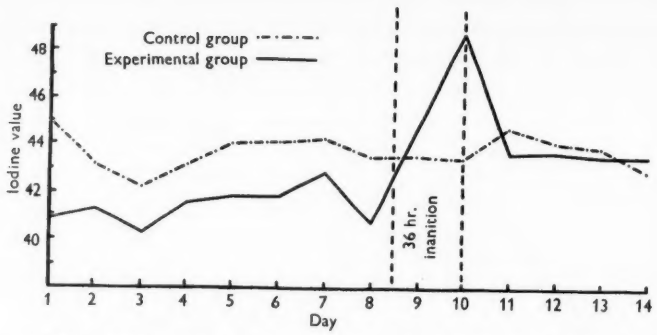


Fig. 4. Effect of 36 hr. partial inanition on iodine values of milk fats of Friesian cows.

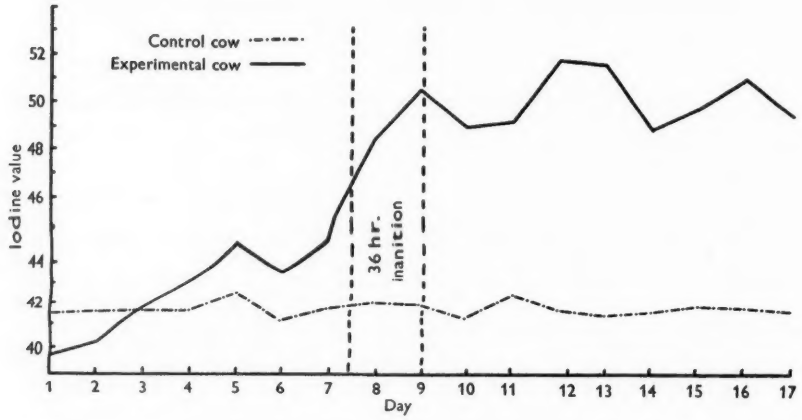
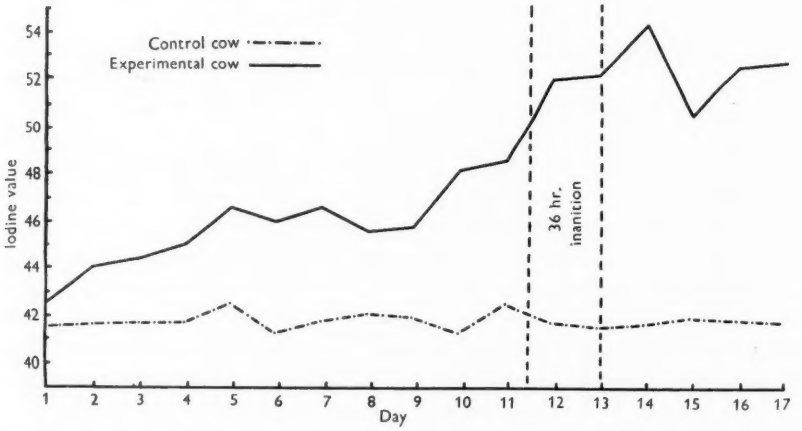


Fig. 5.



Figs. 5 and 6. Effect of 36 hr. partial inanition on iodine values of milk fats of Friesian cows fed 16 oz. peanut oil per day.

(7) *The effect of partial inanition on milk fat composition.* (i) Iodine values of milk fats from daily milk samples of four Friesian cows were followed (Fig. 4) for 15 days. From the eighth day two of the four cows were held for 36 hr. in a yard and deprived of grass, but not of water, and allowed hay and concentrates equivalent to 5·5 lb. S.E. per day—a "maintenance ration". The iodine values of the milk fats of the animals were normal, but the short period of partial inanition raised that of the experimental animals by an average of 5·7 units. (This is the difference between the average of the 12 and 36 hr. values during inanition and the average of the two values 24 hr. before and the two values 48 hr. after inanition.)

(ii) The effect of partial inanition on the iodine value of the milk fat was also studied in animals receiving 16 oz. peanut oil per day. The results are shown in Figs. 5 and 6. During the 36-hr. period of partial inanition hay and meal were given to the experimental animals, the total S.E. of the oil and dry foods being 5·4 lb.—a "maintenance ration". During inanition the increases in iodine value calculated by the foregoing method were only 2·8 and 1·8, average 2·3 units.

INTERPRETATION OF RESULTS

Variations in the iodine value of milk fats are influenced mainly by changes in the oleic acid content (Hilditch (8)). From a study of the analytical results published by Hilditch and co-workers (8) and by McDowell (10) for 32 milk fats in which the iodine value varied from 31·6 to 45·9 with an average of 37·0, it was found that there was a highly significant correlation between the percentage of oleic acid in the fats and their iodine values ($r = 0{\cdot}93$). Calculations showed that 87 % of the variations in iodine value were associated with variations in their oleic acid content. It was concluded that the content of oleic acid in milk fat can be estimated with a reasonable degree of accuracy by multiplying the iodine value by 0·90.

Since it has been shown by Hilditch (8) that the unsaturated acids ingested in peanut oil appear chiefly as oleic acid in the milk fat, the uptakes of unsaturated acids in these experiments have been calculated from the increases in the iodine value of the milk fat, on the assumption that (i) increases in iodine value of milk fat are due to increases in the oleic acid content, (ii) the oleic acid incorporated is derived from that originally present in the oil and from that made in the animal from the more highly unsaturated long-chain acids of the oil, and (iii) other biochemical transformations of the ingested fatty acids (Schoenheimer (16)) are of minor importance quantitatively.

From the analysis of the peanut oil used in this work, its composition was calculated as 44·5 % oleic acid, 31·0 % linoleic acid and 19·5 % saturated acids, the remainder being glyceride residue and unsaponifiable matter. There was only a trace of triene acids. If therefore the oil was saturated to the monoethenoid stage it would contain a total of 75·5 % "oleic acid" (44·5 + 31·0). The iodine value of the original oil was found to be 96·2 and by calculation the iodine value of the oil containing 75·5 % 'oleic acid' would be 67·8.

The extent to which dietary fatty acids were incorporated into milk fat was estimated from increases in its iodine value relative to that of the control milk fats during the period of experimental feeding. By measuring differences in this manner, the influence of individuality and of seasonal trends are compensated for (Table 2).

In Figs. 5 and 6 it is seen that the increases in iodine values before partial inanition ($6\frac{1}{2}$ and $10\frac{1}{2}$ days after commencing oil feeding) were 6·0 and 7·2, average 6·6 units. After the 36-hr. period of partial inanition the values were, on the average, 2·3 units higher than before. An increase in iodine values after resuming normal feeding is also evident in the results of the other inanition experiment (Fig. 4).

Table 2. *Summary of experimental results*

Fig. no.	Oil fed per day (oz.)	No. of cows: Experimental	No. of cows: Controls	Average 'basic' iodine value of controls	Increase in iodine value due to oil-feeding	Average daily yield of milk fats (oz.)
2 (Jerseys)	8	3	3	30·1	4·4	23·2
5 and 6 (av.) (Friesian)	16 (before inanition)	2	1	41·7	6·6	14·4
	16 (after inanition)	2	1	40·9	9·4	—*

* Not measured, but even less than 14·4 oz. because of combined effects of inanition and seasonal trends.

On the assumption that increases in iodine value of milk fat are derived from the 'potential oleic acid' of the ingested oil, and that only the ingested acids more unsaturated than those of the milk fat raise its iodine value, the estimated amounts of ingested oil incorporated into milk fat are as shown in Table 3.

Table 3. *Estimated amount of ingested oil incorporated into milk fat*

Oil fed/day (oz.)	'Potential oleic acid' fed per day (oz.)	Estimated weight of oleic acid in milk fat derived from ingested oil (oz.)*	Estimated percentage of unsaturated acids retained in milk fat
8	6·05	2·25	37
16 (before inanition)	12·1	3·65	30
16 (after inanition)	12·1	5·30	43

* Calculated from the ratios between the actual increase in iodine value of the milk fat due to oil feeding and the maximum potential increase:

$$\frac{\text{Increase in iodine value due to oil feeding}}{\text{'Oleic acid' value of oil fed, 67·8 (see p. 243)} - \text{'basic' iodine value of milk fat (i.e. before oil feeding)}} \times \text{weight of milk fat secreted.}$$

These results indicate that about one third of the dietary long chain unsaturated acids were converted into milk fat. It is further estimated that 30–60% of the unsaturated acids in milk fat were derived from those ingested in the oil.

DISCUSSION

It is shown that iodine values of milk fats are in large measure dependent on their oleic acid contents. Considerable variations in iodine values of milk fats of dairy cows maintained under liberal conditions of grazing were found, e.g. during 6 weeks' observations on ten cows (six Jerseys and four Friesians), extremes were 26·9 (a Jersey) and 45·0 (a Friesian), a range of 18·1 units.

Among the factors influencing the iodine value of milk fat are breed and individuality, and there are others causing regular diurnal and seasonal trends. Diurnal variations

presumably arise from differences in day and night feeding habits of grazing cows. Seasonal trends appear to be physiological (i.e. stage of lactation) in origin, but they are modified by climate and the nature of the food (Cox & McDowall(2)). There are also day-to-day variations of an irregular nature which may be the result of weather changes, either direct on the animal itself or through indirect effects on the nutritive value of herbage.

Dietary factors other than energy or fat intake also promote variations in iodine values of milk fats of grazing cows. McDowell & McDowall(11) have shown that in New Zealand commercial butterfats a parallelism exists between vitamin A potencies and iodine values. McGillivray(12) demonstrated a significant correlation between their vitamin A potency and tocopherol contents, which suggested a relationship between tocopherol and oleic acid contents, since iodine values of milk fats of grazing cows can be raised slightly by feeding tocopherol. Worker(22) showed that botanical composition of pasture is of importance, milk fat from clover-fed cows having a lower iodine value than that from cows on ryegrass.

Partial inanition for 36 hr. during which cows were allowed a 'maintenance ration' and water, caused a sudden rise in the iodine value of the milk fats. The explanation usually given for this phenomenon is that the milk fat synthesized during inanition is at the expense of body fat reserves which have a higher iodine value (and oleic acid content) than milk fat (Palmer & Eckles (quoted by Espe(4)); Riddet, Campbell, Cox & McDowall(15)). According to these workers the rise in iodine value of milk fat brought about by inanition is the result of increased utilization of oleic acid, which Hilditch(8) and Brooker & Shorland(1) have shown to be the predominant unsaturated acid in body fat reserves. It is also generally found that an increase in iodine value is accompanied by a decrease in the content of 'volatile acids' and vice versa.

This theory was tested by observing increases in iodine values of milk fats resulting from partial inanition of cows fed a highly-unsaturated, easily assimilated vegetable (peanut) oil. During the partial inanition (36 hr.), control cows were fed a 'maintenance ration' of hay and meals, and the experimental animals were allowed dry feed to bring their intake of energy to that of the control group. It is seen in Table 3 that prior to inanition about one third of the 'potential oleic acid' of the peanut oil was converted to milk fat. According to the theory of Palmer & Eckles, cited by Espe(4) and later workers, the unsaturated acids of peanut oil should be increasingly utilized during partial inanition and since these acids are considerably more unsaturated than body reserve fats, the ensuing rise in iodine values would be even more pronounced than usual. The increase in iodine value due to inanition (Fig. 4) when no oil was fed was 5·7 units, but when oil was fed it amounted to only 2·3 units (Figs. 5 and 6). This result was unexpected and it does not accord with the hypothesis that the rise in iodine value of milk fats of dairy cows on a lowered plane of nutrition is due to the increased mobilization and utilization of body fat reserves for milk fat synthesis. From these results it would appear that the unsaturated acids of the ingested oil were not removed by the gland to compensate for the decreased supply of the normal precursors of milk fat. Moreover, it is evident (Table 3) that less than one half of the ingested unsaturated acids were utilized for milk fat synthesis—i.e. other tissues competed successfully for them.

It is estimated (Table 3) that about one third by weight of the milk fat of grazing cows was derived from the dietary long-chain fatty acids. It is further deduced that milk fat

of (maximum) iodine value of 50·3 from a cow fed 16 oz. peanut oil per day, had derived 60% by weight of its unsaturated acids from the oil. This finding supports that of Duncombe & Glascock (3) who showed by means of isotope studies with a lactating goat that about 70% of the labelled dietary long-chain acids were secreted in milk fat within 5 days of a single dosing.

It is now generally accepted that the lower molecular weight acids of ruminant milk fats are derived chiefly from short-chain acids produced in the rumen by microbial fermentation of carbohydrates (Popjak (14)), but it appears that the main source of C_{18} acids is the blood glycerides (Folley (5)). It would seem also that dietary fats are an important source of these acids of cow milk fat. Although these C_{18} acids are readily synthesized from non-fat substances of the diet, it has been shown by a number of workers (references in Maynard (13)) that lactating cows yield better when their diets contain a small proportion (5% or more) of fat.

Results obtained in this laboratory and also by Sears, Goodall & Coop (17) and by Sill & Sears (20) indicate that the mixed pastures of New Zealand dairy farms, predominantly perennial rye-grass (*Lolium perenne*) and white clover (*Trifolium repens*) contain 4–6% of ether extract in the dry matter, and about 2·5% of C_{18} highly unsaturated acids (1, 9). Presumably most of these acids are hydrogenated in the rumen (8*a*) chiefly to the monoethenoid stage, since diene and triene acids occur to only a minor extent in milk fat of grazing cows (18). Under New Zealand conditions of grassland farming dairy cows eat, on the average not less than 30 lb. of herbage dry matter per day containing 12 oz. or more of C_{18} unsaturated fatty acids. It is concluded that the assimilation of these amounts of fatty acid could materially influence both composition and yield of milk fat.

SUMMARY

Iodine values of milk fats of grazing cows show wide variations which are chiefly determined by differences in oleic acid content. Among the factors promoting the variations are: individuality of the cow, stage of lactation, breed differences, diurnal and incidental (irregular) day-to-day effects, nature of the food and plane of nutrition.

Results of experiments with feeding an unsaturated vegetable oil indicate that considerable proportions of the unsaturated long chain acids of milk fat were derived from the ingested oil. It is deduced that herbage lipids may contribute materially to the production of milk fat of grazing cows.

From comparisons of the extents to which dietary long chain unsaturated acids are incorporated into milk fats of grazing and semi-fasting cows, it would appear that the rise in iodine value following partial inanition does not result from increased uptake of fatty acids withdrawn from the more highly unsaturated glycerides of the body reserves.

The authors are grateful to Prof. W. Riddet for making available animals used in this investigation; and to Dr F. B. Shorland, Fats Research Laboratory, for the analysis of the oil used in these experiments.

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632. REACTIVATION OF MILK PHOSPHATASE FOLLOWING HEAT TREATMENT

IV. THE INFLUENCE OF CERTAIN METALLIC IONS

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(With 1 Figure)

In an earlier publication (1) it was stated that the reactivation of milk phosphatase following heat treatment was not affected by the presence of ions of Ca or Mg in concentrations likely to occur as contaminants. It has since been suggested by Fuchs (2) that the reactivation of milk phosphatase occurring in 'uperized' milk is due to the activating influence of ions of Mg, Zn and Fe present as contaminants. Whilst this would appear to be an unlikely explanation, particularly in view of the ease with which reactivation can be brought about after heat treatment of milk in glass capillaries where the possibility of metallic contamination cannot arise, further work on this aspect has been carried out and forms the subject of this paper.

THE EFFECT OF METALLIC IONS UPON REACTIVATION OF MILK PHOSPHATASE

Experiments with raw milk

Varying concentrations of these ions were added to raw milk, the milks then heated in sealed capillaries at 95° C. for 30 sec., stored at 26° C. for 18 hr. prior to testing by the modified technique described earlier (3). Table 1 shows typical results which, as previously, are expressed as p.p.m. chlorine, since the permanent colour standards used in the micro-phosphatase test (3) are calibrated in these units. Throughout this work the ions concerned were derived from solutions of the respective sulphates or chlorides. These results indicate that Zn^{2+} has a marked activating effect up to 100 p.p.m., whereas the activating effect of Mg^{2+} only becomes marked above 100 p.p.m. Fe^{2+} is only slightly activating, whereas Ni^{2+} and Sn^{2+} have an inhibitory effect, though this is only slight in the case of Sn^{2+}. The inhibitory effect of Cu^{2+} has been reported previously (1) and has been confirmed, though the actual concentration necessary to inhibit reactivation completely may be as high as 15 p.p.m.

Table 1

	Concentration of ions in p.p.m.							Control
Ion	300	100	50	20	10	5	1	nil
Mg^{2+}	0·6	0·5	0·4+	0·4	0·4	0·4	0·4	0·4
Zn^{2+}	0·2	0·5−	0·6+	0·75	0·8−	0·75	0·6+	0·4
Fe^{2+}	0·4+	0·5	0·4	0·4	0·4	0·4	0·4	0·4
Ni^{2+}	—	—	—	0·1−	0·1+	0·2+	0·4	0·5−
Sn^{2+}	—	0·4+	0·6+	—	0·7−	—	0·6−	0·6

Before proceeding further it was necessary to determine if any of the results illustrated in Table 1 were due to the effect of the ion upon the rate of hydrolysis of the substrate

during the phosphatase test. Many experiments to test this point have been made, but no such effect has been found except in the case of Mg^{2+}, where only a very slight increase has been observed.

Experiments with raw cream (50% *butter fat approx.*)

It has been found that reactivation of phosphatase may occur in cream following heat treatment, the level of reactivation depending upon the method of preparation of the cream, cream prepared by laboratory centrifuging being considerably more easily reactivated than cream produced from an open-type separator. In general, cream reactivates more readily than milk.

The effect of added ions was found to be much the same as with milk. The onset of detectable reactivation, however, occurs more rapidly with cream, and the effect of Mg^{2+} in enhancing this is shown in Table 2 which gives the results obtained when cream prepared from raw milk by laboratory centrifuging was heat treated in capillaries at 135° C. for 20 sec., cooled and then stored at 37° C. It was also found that the same results were obtained if the ion was added after the heat treatment but prior to storage.

Table 2

Storage time	Cream without added ions	Cream + 300 p.p.m. Mg^{2+}
Nil	Nil	Nil
15 min.	0·15	0·4⁻
1 hr.	0·3⁻	1·0⁻
2¼ hr.	0·4+	1·2

The effect of mixtures of ions upon reactivation

It was felt of interest to examine the effect of the addition of an inhibitory ion such as Cu^{2+} or Ni^{2+} upon reactivation of cream shocked in the presence of an activating ion such as Mg^{2+} or Zn^{2+}. Experiments showed that the activating influence of Zn^{2+} was not affected by the presence of either Cu^{2+} or Ni^{2+}, whereas these ions inhibited the activating influence of Mg^{2+}. This effect was observed both when the addition of the inhibitory ion was made immediately after shock treatment and also after a short period of storage at 37° C. during which time some reactivation occurred. Figures illustrating this latter

Table 3

	After 25 min. storage	
	A/M 2 hr.	After further storage 1 hr. 37° C. A/M 2 hr.
Cream + Mg^{2+}	0·5	1·0
Cream + Mg^{2+} + 10% H_2O	0·5⁻	1·0
Cream + Mg^{2+} + Cu^{2+}	0·45	0·45

A/M = Aschaffenburg & Mullen.

effect are shown in Table 3, and were obtained by the addition of 6 p.p.m. Cu^{2+} to shocked cream containing 300 p.p.m. Mg^{2+} after the latter had been stored for 25 min. at 37° C. (1 part ion solution + 9 parts cream).

This difference in the activating influence of Mg^{2+} and Zn^{2+} was confirmed by experiments carried out in the reverse order, i.e. reactivation occurred when Zn^{2+} was added to shocked cream containing either Ni^{2+} or Cu^{2+}, but not when Mg^{2+} was added.

Experiments with water-washed cream

When washed with water or even when mixed with an equal volume of water, cream (butterfat $< 50\%$) usually showed no reactivation, and accordingly water-washed cream has been used in order to determine what factors influence reactivation.

Water-washed cream was prepared as follows:

About 5 g. of cream were mixed with 75 ml. of distilled water and the mixture centrifuged for 10 min. at 2000 r.p.m. The cream was removed, mixed with a further 75 ml. of distilled water and again centrifuged. This process was repeated a third time. Since cream (butterfat $< 50\%$) contains 5–7 times as much phosphatase as the corresponding raw milk, and since the above washing treatment only reduces the phosphatase content by one-half, the failure of such washed cream to reactivate cannot be attributed to a low concentration of the enzyme.

This failure to reactivate cannot be attributed to the absence of Mg^{2+} in the washed cream causing a negative result to be obtained in the phosphatase test. The results shown in Table 4 were obtained using cream from underheated milk and washed in the manner described above. These figures show that the rate of hydrolysis of the substrate is almost unaffected by the addition of Mg^{2+}.

Table 4

	Mg^{2+} in mixture p.p.m.	A/M test readings		
		10 min.	20 min.	30 min.
Washed cream + 10% H_2O	Nil	0·3+	0·45	0·5+
Washed cream	300	0·3+	0·5−	0·6−
Washed cream	740	0·3+	0·45	0·5+

Reactivation of water-washed cream can be brought about by the addition of whey either before or immediately after shock treatment, and further work on the effect of the addition of various whey fractions showed that the ash of whey would bring about reactivation of washed cream. This effect was confined to the water-insoluble constituents, and addition of the common constituents of the insoluble ash to washed cream showed that only salts of Mg were able to bring about reactivation.

On a few occasions the addition of Mg^{2+} has failed to bring about reactivation of washed cream, but it is of interest to note that though Mg^{2+} usually induces reactivation, the addition of Zn^{2+} has always failed to cause reactivation to take place.

Resin-treated whey

In view of this positive effect of Mg^{2+}, experiments were made using whey after treatment with cationic resins. Such wheys were prepared as follows:

Rennet whey was acidified with trichloracetic acid to pH 4·0 approximately, boiled for 5 min. and then filtered through Kieselgur. After cooling, 16·5 g. Amberlite Resin IR–120 (H) were added per 100 g. whey and the mixture stirred for 1 hr. The whey was then decanted and the pH adjusted to 6·5 approx. with NaOH.

Portions of this treated whey, to which varying amounts of Mg^{2+} had been added, were mixed with washed cream, these mixtures then being heat treated at 95° C. for 30 sec. Similar concentrations of Mg^{2+} in distilled water were mixed with washed cream prior to heat treatment. The results of one such experiment are shown in Table 5.

Table 5

	Mg^{2+} in mixture (p.p.m.)	% CW in mixture	A/M test after storage
1. CW only	—	100	Nil
2. CW + resin-treated whey	—	66	Nil
3. CW + resin-treated whey + Mg^{2+}	100	63	1·25
4. CW + resin-treated whey + Mg^{2+}	45	55	1·25
5. CW + resin-treated whey + Mg^{2+}	12	65	0·8⁻
6. CW + distilled water + Mg^{2+}	260	91	0·5
7. CW + distilled water + Mg^{2+}	110	63	0·2
8. CW + distilled water + Mg^{2+}	37	63	0·2⁻
9. CW + rennet whey	—	—	0·8
10. CW + rennet whey after treatment at pH 4·0	—	—	0·6

CW = washed cream.

These results show that whilst treatment of whey by boiling at pH 4·0 only slightly reduces its ability to bring about reactivation of water-washed cream, further treatment by passage through a cationic resin completely removes this property. The addition of Mg^{2+} restores this property even at such a low concentration as 12 p.p.m. It will be noted that much less reactivation is obtained using Mg^{2+} in distilled water.

The effect of the addition of Mn^{2+} and Zn^{2+} to such resin-treated wheys has also been examined, and whilst the former behaved in a similar manner to Mg^{2+}, the addition of Zn^{2+} failed to bring about any reactivation. Thus the difference in the role of Mg^{2+} and Zn^{2+} in reactivation is again apparent.

In these experiments, both with acid-treated whey and with such whey after passage through the cationic resin, it is emphasized that the pH of the whey must be adjusted to 6·5 approx. before mixing with washed cream. This is essential because we have found that reactivation is dependent upon pH, being optimal about 6·5 and falling to a zero value at pH's of 5·5 and 8·3.

The effect of metallic ion chelating agents upon reactivation of cream

In view of the work of Schwarzenbach and others on the formation of unionized metallic complexes with such substances as the disodium salt of ethylene diamine tetracetic acid (EDTA), experiments have been made using this substance to investigate the possible role of metallic ions on reactivation. Solutions of the salt* used in this work were standardized against standard Ca solutions.

Varying concentrations of EDTA were added to raw cream by weight in the proportion of 1 part EDTA to 9 parts cream and the mixtures heat treated at 95° C. for 30 sec. in capillaries, which were examined by the A/M test after storage for 18 hr. at 26° C. The results are shown in Table 6.

These results show that the degree of reactivation obtained is inversely proportional to the amount of EDTA present. The possibility that the presence of EDTA interferes with the actual phosphatase test was next examined. Using under-pasteurized cream and

* Ex General Chemical and Pharmaceutical Co. Ltd., Sudbury, Middlesex.

milk containing sufficient residual phosphatase to give A/M test readings of the same order as the above controls (i.e. 0·8 approx.), the presence of EDTA in a concentration of 1×10^{-3} M had no effect upon the test reading, even after storage at 26° C. for 18 hr prior to testing. The inhibitory effect upon reactivation cannot therefore be ascribed to any interference in the actual phosphatase test.

Table 6

Concentration of EDTA in cream	Reactivation A/M test
1×10^{-3} M	Nil
$3{\cdot}3 \times 10^{-4}$ M	Nil
$1{\cdot}1 \times 10^{-4}$ M	0·1
$3{\cdot}7 \times 10^{-5}$ M	0·2+
$1{\cdot}7 \times 10^{-5}$ M	0·5
$4{\cdot}1 \times 10^{-6}$ M	0·75
Nil	0·8
Nil + 10% water	0·75

In order to study further this inhibitory action, cream (butterfat 50% approx.) was titrated against M/100-EDTA in the presence of NH_4OH/NH_4Cl buffer with Eriochrome Black T as indicator, which was the procedure used when standardizing the EDTA against standard Ca solutions. The average titration of such cream was found to be 10 ml/10 g. Since the addition of 10% of a M/200-EDTA solution to cream has always been found to inhibit reactivation, this amount is only $\frac{1}{20}$th of the amount necessary to complex the available ions in the cream, which are assumed to comprise mainly Ca^{2+} and Mg^{2+}. This suggests the possibility of the inhibition being due to the complexing of other ions, and accordingly further experiments have been made using mixtures of M/100-EDTA solution and M/100 solutions of various metallic ions, in order to examine the effect of using EDTA solutions in which the chelating power has been partially or completely satisfied by metallic ions. The results obtained are illustrated in Fig. 1 (1 part EDTA solution + 9 parts cream).

In considering these results the possible effect of pH must be borne in mind, not only because of its effect on reactivation, but also because of the relationship between pH and the chelating effect of EDTA. As far as the former is concerned, no evidence has been found that the curves in Fig. 1 have been influenced by varying pH, both by direct pH measurement and by suitable adjustment. In this connexion, the M/100-EDTA solution was always adjusted to pH 6·5 by addition of NaOH and thus contained a mixture of the di- and tri-sodium salt.

Fig. 1 shows that for two inhibitory ions, Co^{2+} and Ni^{2+}, the inhibitory effect of EDTA is largely eliminated by approximately 50/50 mixtures. In the presence of excess of either the ion or EDTA, reactivation lessens as expected, and it will be noted that the concentration of free EDTA at the point where reactivation becomes zero agrees with the value shown in Table 6. The use of two activating ions, Zn^{2+} and Mg^{2+}, led to very different results, the Zn^{2+} curve suggesting that the 50/50 mixture contained free Zn^{2+} having their expected activating effect, the 'neutral' mixture being a 43/57 mixture of Zn^{2+} and EDTA respectively. The Mg^{2+} curve, on the other hand, falls away rapidly to a nil value at an EDTA concentration of $1\text{–}2 \times 10^{-4}$ M, which from Table 6 suggests that all the EDTA in the mixture is acting as free EDTA. No curve has been included for the inhibitory Cu^{2+} ion since, using M/100 solutions, it has not been possible to obtain any reactivation.

Weaker solutions of EDTA and Cu^{2+} suggest that, as with Mg^{2+}, all the EDTA and/or Cu^{2+} in the mixture is free, which is contrary to published figures for stability constants of these metal complexes.

The results are referred to again under 'Discussion'. It is of interest to record here that no effect upon reactivation has been observed using an Fe specific versene.

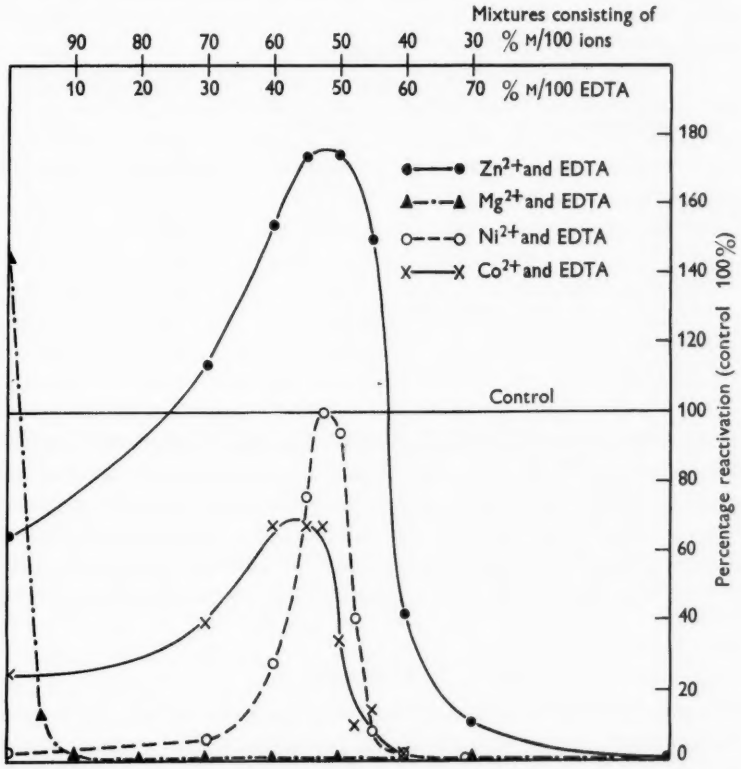

Fig. 1. Effect of adding mixtures of EDTA and some metallic ions on the reactivation of phosphatase in cream

The effect of EDTA upon raw-milk phosphatase

It has already been stated that the presence of EDTA in a concentration of 1×10^{-3} M has no effect upon phosphatase present in either under-pasteurized cream or milk, even after overnight storage. It was felt of interest, however, to determine the concentration of EDTA required to inactivate phosphatase in cream and water-washed cream. For this purpose cream was prepared from under-pasteurized milk, a portion water-washed and varying concentrations of EDTA added to both. The phosphatase test was then carried out both immediately and after storage for 18 hr. at room temperature. In addition, the effect of adding mixtures of equal volumes of M/100-EDTA and various M/100-ion solutions to water-washed cream was also observed, the pH of the mixtures being adjusted to 6·5 before addition to the water-washed cream in view of the absence of buffering power in such cream. The results are given in Table 7.

It will be seen from Table 7 that considerable inactivation of phosphatase occurs at an EDTA concentration of 3×10^{-2} M in cream, and that a concentration of only 5×10^{-4} M is necessary to bring about a similar degree of inactivation in water-washed cream. It will also be seen that when the EDTA is 'neutralized', with ions such as Ca^{2+}, Mg^{2+} or Zn^{2+} no such inactivation of phosphatase occurs. These results therefore confirm other published observations that a metallic ion is essential for normal alkaline phosphatase

Table 7

Concentration of EDTA in cream	Cream		Water-washed cream	
	Tested immediately	Tested after 18 hr. at room temperature	Tested immediately	Tested after 18 hr. at room temperature
Nil	1·25	1·0+	0·45	0·5−
$1{\cdot}4 \times 10^{-2}$ M	0·9	0·9	Nil	Nil
$2{\cdot}4 \times 10^{-2}$ M	0·7	0·2+	Nil	Nil
$3{\cdot}4 \times 10^{-2}$ M	0·6+	0·1	Nil	Nil
$4{\cdot}4 \times 10^{-4}$ M			0·35	0·1
$6{\cdot}6 \times 10^{-4}$ M			0·2−	0·1
EDTA/Ca	As control values, i.e.		0·5	0·4−
EDTA/Mg	no effect observed		0·6	0·4−
EDTA/Zn (50/50 mixtures 5×10^{-4} M)			0·6	0·6+

activity, the much lower concentration of EDTA required to inactive water-washed cream being due to the removal of serum and consequently of ions such as Ca^{2+} and Mg^{2+} which are chelated by EDTA. At the same time it can be seen that the concentration of EDTA required to inactivate the phosphatase is, in both cases, considerably greater than that required to complex all the available Ca^{2+} and Mg^{2+} as shown by titration at pH 10 approx., (5×10^{-3} M and less than 5×10^{-5} M for cream and water-washed cream respectively).

DISCUSSION

There is much published evidence of the activating influence of certain metallic ions upon alkaline phosphatases. In much of this, phosphatases from sources other than milk have been used, but some observations (4, 5, 6, 11) have been made on the alkaline phosphatase of cow's milk. In general, enzyme preparations have been used, and in many cases evidence of the activating influence of metallic ions has been obtained by observing the loss of activity of such preparations during dialysis and the restoration of activity by the addition of metallic ions such as Mg^{2+}. In addition, Morton's (11) findings must be borne in mind, namely, that the effect of added salts may vary both qualitatively and quantitatively depending on the purity of the enzyme preparation.

In the work reported in this paper, the milk phosphatase used has not apparently been attenuated to any significant extent, for even in water-washed cream no activating effect has been found except with Mg^{2+}, where only a very slight increase has been observed. Nevertheless, our experiments covering the effect of EDTA upon the activity of the raw enzyme confirm the findings of Anagnostopoulos (7) and point to the conclusion that some metallic ion or ions play a part in the normal activity of alkaline phosphatases.

Nearly all the work reported in this paper, however, has been directed towards studying the influence of certain metallic ions upon reactivation of alkaline phosphatase following

heat treatment. The fact that certain ions have been found to be activating and others inhibitory in this connexion is not surprising in view of the essential part played by metallic ions in alkaline phosphatase activity. In studying these results it should be borne in mind (1) that in cream the amount of EDTA required to inhibit reactivation is less than 1% of that required to inhibit the activity of the raw enzyme; (2) that the inhibitory effect of EDTA on reactivation cannot be overcome by 'neutralizing' the EDTA with Mg^{2+} or Ca^{2+}, whereas the inhibitory effect of EDTA upon the activity of the raw enzyme can be overcome by 'neutralizing' the EDTA with either of these ions; (3) that milk contains about 120 p.p.m. magnesium and 1–2 p.p.m. zinc.

It would therefore appear that the process of reactivation may involve a trace metal, and our results suggest that Zn may well be the particular ion involved. For instance, the results illustrated in Fig. 1 are not inconsistent with this view, for the fact that the two inhibitory ions Co^{2+} and Ni^{2+} can counteract the inhibitory effect of EDTA is explained by the published observations that both these ions are complexed more readily than Zn^{2+}. This possible role of Zn^{2+} finds support in the work of Cloetens (8) and Hove, Elvehjem & Hart (9), though other authors have failed to find evidence in favour of this theory.

Attention has already been drawn to differences between the effect of Mg^{2+} and Zn^{2+}, and in particular to the fact that we have been unable to induce reactivation of water-washed cream by Zn^{2+}, either in the presence or absence of resin-treated whey, whereas Mg^{2+} will bring about reactivation in both cases. While Hove *et al.* and Abul-Fadl and King (10) have suggested an organic dialysable substance to be an essential part of alkaline phosphatase, Morton (11) has failed to obtain evidence for this. Since, however, in our experiments, Zn^{2+} has failed to induce reactivation of water-washed cream, it is thought that the lack of such an organic substance may possibly be the explanation. On the other hand, the fact that reactivation occurs with mixtures of water-washed cream and whey ash does not support this view. The necessity for some such organic substance finds further support in, for example, the results in Table 5, which show that small amounts of Mg^{2+} (45 p.p.m.) brought about very good reactivation with mixtures of water-washed cream and resin-treated whey, which was never obtained to a comparable degree by the addition to water-washed cream of a similar concentration of Mg^{2+} in distilled water.

It would seem, therefore, that either Mg^{2+} or Zn^{2+} can take part in the mechanism of reactivation. Since the addition of Mg^{2+} and not Zn^{2+} will result in reactivation after shock treatment of mixtures of water-washed cream and resin-treated whey or even of water-washed cream itself, and bearing in mind that raw washed cream shows normal phosphatase activity, it may be that Zn is an integral part of the active phosphatase complex, whereas Mg is not.

We have not found any evidence that Mg is essential for alkaline milk phosphatase activity, though again Mg may be an integral part of the active phosphatase complex. There is considerable evidence, however, that Mg^{2+} is essential for reactivation to take place, and it may be that Zn^{2+} can act as an alternative ion in this respect.

The effect of Cu^{2+} in being inhibitory towards Mg^{2+}-induced reactivation, but not against that activated by Zn^{2+}, can be explained by assuming that it blocks some essential reaction involving Mg^{2+}, but is unable to do so in the case of Zn^{2+}.

SUMMARY

1. Reactivation of alkaline milk phosphatase is affected by the presence of ions, Mg^{2+}, Zn^{2+} and Mn^{2+} being activating and Cu^{2+}, Ni^{2+} and Co^{2+} being inhibitory.

2. Cu^{2+} will inhibit reactivation induced by Mg^{2+}, but has no effect upon Zn^{2+}-induced reactivation.

3. Using washed cream, either alone or in admixture with boiled whey treated with cationic resin, reactivation occurs in the presence of added Mg^{2+}, but not when Zn^{2+} is added.

4. Experiments with EDTA. confirm that metallic ions play a part both in alkaline phosphatase activity and in reactivation.

5. It is suggested that either Mg^{2+} or Zn^{2+} or both play a part in reactivation.

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633. THE EFFECT OF COMMERCIAL STERILIZATION ON THE NUTRITIVE VALUE OF MILK AS DETERMINED IN EXPERIMENTS WITH RATS AND BABY PIGS

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Because of its excellent keeping qualities the use of sterilized milk is rapidly increasing in several European countries and in the United States, while in other parts of the world, especially those with a warmer climate, considerable interest has developed. Since heat treatment of food products often causes a reduction in nutritive value, it is important to know to what extent the nutritive value of milk is affected by the commercial process of sterilization.

It has been shown by several authors that alterations in the nutritive value of milk during pasteurization are minimal. The more drastic heat treatment necessary for sterilization, however, destroys some of the vitamins and may bring about a reduction in the nutritive value of the milk proteins. Of the thiamine originally present 20–30% may get lost, while vitamin C, which decomposes already during pasteurization, is destroyed for the greater part (Henry & Kon (6, 7); van der Mijll Dekker & Engel (15)). Losses in carotene, vitamin A and riboflavin were not observed by these authors. Henry & Kon (8) obtained a reduction of 6% in the biological value of the milk proteins as a result of sterilization. Schroeder, Iacobellis & Smith (18) in experiments with dogs, and Hodson (10) using the rat repletion method, could not detect a significant deleterious effect of the heat treatment. In growth experiments with rats, Henry, Ikin & Kon (9), and recently Hodson (11), observed a small reduction in total nutritive value of milk as a result of sterilization, whereas Bixby, Bosch, Elvehjem & Swanson (2) found no decrease at all.

The changes occurring in milk submitted to high temperatures are determined primarily by the temperature applied and by the duration of the heat treatment. Consequently the nutritive value will depend on the system of manufacturing. In recent years plants were developed for large-scale production of bottled sterilized milk, not only to secure high output but also to obtain a product with good keeping quality and retaining as far as possible its colour, taste and nutritive value. In the Netherlands the Stork sterilizer was developed. This operates continuously in a two-phase system with one heat treatment prior to bottling and a second thereafter. The nutritive value of milk sterilized in a Stork apparatus was studied by Jochims & Wilckhaus (12) in 477 human babies from birth up to 10 months of age. Though significant differences were not observed, the authors concluded that sterilized milk is not equivalent to pasteurized milk as judged by growth performance, dyspepsiae and tolerance of overfeeding.

In order to obtain exact information on the nutritive value of milk sterilized in the Stork apparatus we started laboratory experiments in 1953. Sterilized milk was compared with pasteurized and raw milk in experiments with albino rats by determining the

biological value, the repletion value for protein-depleted animals and the growth-promoting value. In subsequent growth experiments baby pigs were fed pure milk diets with equalized intake of pasteurized and sterilized milk.

MATERIAL AND METHODS

(*a*) *Milk.* All milk used in the experiments to be reported was obtained from a mixed bulked supply and processed as is usual in the Netherlands for human consumption, viz. standardized at a fat content of 2·5% and bottled. The pasteurized and the sterilized milks were homogenized, the raw milk was not. The three types of milks were prepared from one batch of pooled milk except for some lots used in the rat experiments.

Pasteurized milk was prepared in the Stork tubular pasteurizer and flash heated to 72° C. for 20 sec.

The Stork procedure for large-scale production of sterilized milk consists of two subsequent heat treatments. In the first one the milk is submitted for approx. 15 sec. to a temperature of 135° C. on passing a tubular flow sterilizer which kills all micro-organisms and spores. This pre-sterilized product is bottled and capped after cooling to 70° C. In the second phase a temperature of 113° C. is applied for a longer time (± 15 min.), the intensity of the heat treatment being dependent on amount and heat resistance of the microbes which infect the milk during bottling and capping.

That sterilization was effective was shown at intervals by the procedure developed by Mossel & Drion (16).

The milks processed outside the city of Utrecht were received about once weekly and kept in a refrigerator.

(*b*) *Determinations of the biological value.* The biological values of the milk proteins were determined by the method of Mitchell (14). Three groups of six male rats weighing approximately 200 g. were housed individually in metabolic cages to collect faeces and urine. Each group received the three kinds of milk separated from a protein-free food in successive periods of 7 days. The composition of the protein-free diet is given in Table 1. The milk periods were preceded and followed by a standardizing period, in which only the protein-free food was supplied. During the first 3 days the animals were accustomed to the diet. Urine and faeces were collected during the remaining 4 days of the period. The amounts of protein-free food and milk were regulated in such a way that the animals received about 8·5% of protein in their diet and that nearly all animals consumed all food supplied. The unconsumed food residues were collected daily and dried.

(*c*) *The rat repletion method.* Evaluation of the milk proteins with the rat repletion method (as described by Frost & Sandy (5)) was performed with three groups of eight male rats (weighing approximately 200 g.), housed in single wire cages with screen bottoms and fed a protein-free diet of the following percentage composition: sucrose 83, coconut oil 4·2, mixed vegetable oil 4·6, cod-liver oil 1·5, agar 1·4, minerals (Steenbock 40) 4, $CaHPO_4.2H_2O$ 1·0, and trace minerals and vitamins, as given in Table 1. The non-protein diet and water were supplied *ad libitum*. When about 25% of the body weight was lost (which happened in about 3 weeks) each group was fed one of the three milks separated from the protein-free diet. During 5 days 40 ml. milk were supplied daily. The increase in body weight was determined at the sixth day. Then a stock ration was fed for 2 days followed by a second protein-free period. When the rats had again sufficiently

lost weight, the milk feeding was repeated, each group being fed another kind of milk than in the first milk period. This depletion and repletion was repeated for a third time allowing each rat to consume each of the milks in turn.

Table 1. *Composition of protein-free diet for determinations of the biological value of milk proteins*

Components	Amount (g./100 g.)	Components	Amount (mg./100 g.)
Wheat starch	50	Thiamine	0·6
Potato starch	10	Riboflavin	1·2
Sucrose	18·4	Nicotinic acid	3·7
Lard	15	Pyridoxine HCl	0·6
Cod-liver oil	1·5	Ca-pantothenate	5·0
Salts (Steenbock 40)	4·0	*p*-Aminobenzoic acid	0·6
$CaHPO_4.2H_2O$	1·0	Folic acid	0·6
$MnSO_4.4H_2O$	0·001	Menadion	0·4
$CuSO_4.5H_2O$	0·004	Biotin	0·04
$ZnSO_4.7H_2O$	0·004	Vitamin B_{12}	0·0025
Co-acetate	0·0002	Tocopherol acetate	1·5
		Ascorbic acid	10

Animals which did not consume the 40 ml. of milk were omitted from the calculation.

(*d*) *Growth experiments with rats.* Three groups of twelve male and twelve female weanling rats weighing approximately 50 g. were fed an exclusive milk diet consisting of either raw, pasteurized or sterilized milk supplemented with minerals (per l. of milk 50 mg. $FeSO_4.7H_2O$, 8 mg. $CuSO_4.5H_2O$ and 9 mg. $MnSO_4.4H_2O$). The milk was offered *ad libitum*, but the amounts consumed were recorded daily. The animals were kept in wire cages on screen bottoms (three animals in each cage) and weighed twice weekly. The experiment was continued for 6 weeks.

(*e*) *Growth experiments with baby pigs.* Baby pigs from 1 to 4 days of age were housed individually in metal cages with raised screen bottom, and kept in an electrically heated room at about 23° C. Heat lamps supplied additional heat during the first 4 weeks. The animals available for each experiment were divided in two comparable groups, based on litter, weight and sex and fed on either pasteurized or sterilized milk with only minerals added (per l. of milk 50 mg. $FeSO_4.7H_2O$, 8 mg. $CuSO_4.5H_2O$ and 9 mg. $MnSO_4.4H_2O$). Four times a day each animal received an amount of milk carefully measured in a graduated cylinder. The amount supplied was increased according to appetite in such a way that all animals consumed equal amounts of milk which were readily drunk within 30 min. When the milk consumption had risen to 500 ml. per feeding (which happened after 3–4 weeks) a further increase in appetite was covered by adding to the milk increasing amounts of a meal mixture of the following percentage composition: fish meal 10, skimmed-milk powder 10, ground corn 20, ground dehulled oats 30, ground wheat 19·6, linseed-oil meal 5, vitamin B preparation (Vital) 4, minerals 1, vitamin AD_2 oil 0·4. An increase of total solids above 20% as a result of the added pig starter was prevented by adding tap water which dispensed with the necessity of an additional source of drinking water.

Male pigs were not castrated until the end of the experiment.

RESULTS

(1) *The biological values and digestibilities of raw, pasteurized and sterilized milk*

The figures obtained with each rat in three successive experimental periods are summarized in Table 2 together with the corresponding mean values. The values found in successive periods do not agree in every respect. In the first period the means of the biological values are markedly lower than in both other periods, the figure for raw milk being especially divergent. The mean biological value of sterilized milk is lower than that for raw and pasteurized milk in the first and second period. In the third period the values are practically equal.

Table 2. *Biological values and true digestibilities of raw, pasteurized and sterilized milk obtained in three subsequent experimental periods*

Period	...	1			2			3		
Milk	...	Raw	Past.	Ster.	Raw	Past.	Ster.	Raw	Past.	Ster.
Group of rats	...	B	A	C	A	C	B	C	B	A
					Biological values					
		81	84	83	90	91	83	94	92	89
		83	95	83	96	86	81	86	90	99
		78	84	77	86	84	84	86	89	86
		86	83	84	86	93	90	95	89	86
		81	85	73	84	92	81	90	84	90
		85	87	79	93	85	90	86	92	95
Mean		82·3	86·3	79·8	89·2	88·5	84·8	89·5	89·3	90·8
					True digestibilities					
		100	97	92	95	95	100	92	97	92
		95	100	95	99	100	97	97	97	99
		94	92	97	97	100	97	95	98	92
		100	95	96	99	100	97	100	95	89
		100	97	87	100	91	95	97	94	99
		92	100	89	99	92	96	92	94	91
Mean		96·8	96·8	92·7	98·2	96·3	97·0	95·5	95·8	93·7

The figures obtained for digestibility of sterilized milk are somewhat lower than those for raw and pasteurized milk, except in the second period.

On pooling the data of the three periods mean biological values of 87·0, 88·0 and 85·1 are obtained for raw, pasteurized and sterilized milk respectively, while the corresponding digestibilities are 96·8, 96·3 and 94·4.

In general, these values are in good agreement with those reported by Henry & Kon (8), who obtained a biological value and true digestibility of 84·3 and 96·4 respectively for raw milk, and of 79·1 and 95·3 for commercially sterilized milk. The differences in biological value and digestibility obtained in the present experiment are statistically not significant according to the test of Wilcoxon ($P > 0{\cdot}01$).

(2) *The relative nutritive values of raw, pasteurized and sterilized milk for repletion of protein-depleted rats*

The weight increments of the individual rats which consumed all milk supplied on 5 subsequent days are given in Table 3, together with their mean values. The average figures obtained in three successive experimental periods show a quite uniform picture, though in general the weight gains decrease gradually. In each experimental period the

average weight gain was highest for raw and lowest for sterilized milk, the value for pasteurized milk being intermediate. The difference in weight gain on sterilized and pasteurized milk is significant only in the first period ($P = 0{\cdot}01$). The mean values obtained on pooling all figures for raw, pasteurized and sterilized milk are 27·7, 26·3 and 22·9 g. respectively. The difference of 3·4 g. between pasteurized and sterilized milk is statistically not significant ($P = 0{\cdot}06$).

Table 3. *Individual and average weight (in g.) of depleted rats after feeding raw, pasteurized and sterilized milk (40 ml. per rat per day) during 5 days obtained in three subsequent repletion periods*

Period	...	1			2			3		
Milk	...	Raw	Past.	Ster.	Raw	Past.	Ster.	Raw	Past.	Ster.
Group of rats	...	*b*	*c*	*a*	*c*	*a*	*b*	*a*	*b*	*c*
		—	38	28	25	27	—	20	20	19
		—	—	29	23	30	15	28	21	20
		37	32	18	26	14	28	17	24	19
		39	34	29	27	25	—	20	—	25
		31	—	26	25	27	23	24	23	21
		31	26	25	27	23	23	28	22	20
		29	33	23	30	23	23	21	23	24
		37	31	29	28	27	26	24	21	11
Mean		34·0	32·3	25·9	26·4	24·5	23·0	22·8	22·0	19·9

(3) *Growth experiment with rats on raw, pasteurized and sterilized milk*

The weight increments and amounts of milk consumed are summarized in Table 4. During the first 2 weeks of milk feeding, diarrhoea occurred in all groups, which decreased gradually but did not disappear completely. In this respect no difference was noticeable between the three groups. No animals died during the experimental period.

The mean gains in weight of rats fed the three types of milk are very similar in both sexes. The male rats fed sterilized milk gained somewhat more than did those fed pasteurized milk. For female rats the reverse occurred. These differences are statistically of no importance.

Male and female rats consumed less raw than either pasteurized or sterilized milk. This may be caused by more rapid souring of raw milk in the food containers. Male rats consumed more sterilized than pasteurized milk. For female rats the difference is negligible.

Male and female rats fed raw milk gained more per g. of solids consumed than did rats fed either pasteurized or sterilized milk. In this respect no distinct difference was noticeable between pasteurized and sterilized milk.

The higher intake and better food efficiency obtained on raw milk is not in agreement with findings of Bartlett, Henry & Kon (1), who obtained no difference between raw and pasteurized milk in these respects.

Parallel groups of rats fed the three types of milk but supplemented with either thiamine (0·2 mg/l.) or vitamin B_{12} (0·01 mg./l.) showed no apparent differences with groups on the non-supplemented milks with respect to growth, milk consumption and efficiency of food utilization. The relevant figures have therefore not been given in Table 4.

The rate of growth obtained in the present experiment is considerably less than that

normally found on our stock ration. Calculated over a period of 6 weeks from weaning we usually obtain a daily growth increment of at least 4 g. for males and 3 g. for females on our stock ration, while the corresponding figures of Table 4 are much lower. It is not unlikely that small differences in the nutritive value of the milks tested were masked by the subnormal growth rate. In as yet unpublished experiments from this laboratory it was shown that the poor growth rate may be ascribed to the high lactose and water content of the cow's milk. Obviously our rats are not ideal experimental animals for testing the total nutritive value of milk. Rapidly growing animals like the baby pig are particularly sensitive to food deficiencies. As it was known from literature that baby pigs thrive on a diet of cow's milk only, these animals were considered to be more suitable to detect small nutritional differences in cow's milk.

Table 4. *Mean figures for weight gain and food consumption of male and female rats fed mineralized raw, pasteurized and sterilized milk during 42 days*

Milk	Sex	Weight (g.) Initial	Final	Increase	Increase per day (g.)	Milk intake (ml.)	Solid* intake (g.)	g. gain per g. solid intake
Raw	Male	38·9	157·2	118·3	2·82	2492	289	0·409
Pasteurized	Male	39·2	154·2	115·0	2·74	2651	308	0·373
Sterilized	Male	38·7	158·0	119·3	2·84	2814	324	0·368
Raw	Female	35·0	121·3	86·3	2·05	2011	233	0·370
Pasteurized	Female	34·7	123·8	89·1	2·12	2358	274	0·325
Sterilized	Female	34·9	120·3	85·4	2·03	2377	273	0·313

* Total solids in raw and pasteurized milk 11·6% in sterilized milk 11·5%.

(4) *Growth experiments with baby pigs fed pasteurized or sterilized milk*

(*a*) *First experiment*

A litter of ten pigs, two males and eight females, removed from the dam 2 days after farrowing, were divided equally into two groups and fed gradually increasing amounts of mineralized sterilized and pasteurized milk with equal small supplements of solid food in the last 2 weeks of the experiment.

In this experiment no animals died. However, during the first 2 weeks two pigs on pasteurized and four on sterilized milk suffered from intestinal disturbances as shown by severe scouring, which disappeared gradually without treatment. In this period the sterilized milk was somewhat less rapidly consumed than the pasteurized milk. Thereafter no difference was noticeable in this respect.

In Table 5 individual and mean gains in weight are given, obtained in subsequent weekly periods. It may be seen in this table that the sterilized milk group gained less than the pasteurized group, the difference being important only in the first week. Calculated over each of the five subsequent weekly periods growth on sterilized milk amounted to 79, 98, 99, 98 and 96% of that on pasteurized milk, while for total weight gain this figure is 97%.

(*b*) *Second experiment*

A litter of five male and five female pigs was available for a second experiment 3 days after birth. After dividing into two comparable groups the animals were fed either pasteurized or sterilized milk during 4 weeks. Then the milk was supplemented with the pig starter and the experiment continued for another 2 weeks.

During the first 2 weeks a high incidence of scouring occurred in both groups. All animals exhibited unthriftiness and poor appetite. Not all milk supplied was consumed and consequently food intake of both groups was not equal. Four pigs died, viz. two on pasteurized and two on sterilized milk. The surviving six animals recovered at the end of the second week except one on sterilized milk (no. 17), which produced normal faeces only from the twenty-third day onward.

Table 5. *Initial and final weight (in g.) and increase in weight in successive weekly periods of baby pigs fed on pasteurized or sterilized milk. First experiment*

Experimental animal			Weight		Weight gain per week				
No.	Sex	Milk	Initial	Final	1st	2nd	3rd	4th	5th
1	♀	Pasteurized	1760	9510	370	970	1580	1930	2900
6	♀	Pasteurized	1910	9620	460	940	1590	2070	2650
8	♀	Pasteurized	1850	9670	460	930	1600	1960	2870
10	♀	Pasteurized	1490	9200	370	1020	1430	1930	2960
9	♂	Pasteurized	1790	9890	510	970	1690	2070	2860
Mean			1760	9578	434	966	1578	1992	2848
2	♀	Sterilized	1720	9260	380	860	1510	1990	2800
4	♀	Sterilized	1830	9270	280	980	1590	1930	2660
5	♀	Sterilized	1660	8910	270	930	1500	1860	2690
7	♀	Sterilized	1810	9700	320	1060	1630	1990	2890
3	♂	Sterilized	1690	9290	460	900	1560	2040	2640
Mean			1742	9286	342	946	1558	1962	2736

Table 6. *Initial and final weight (in g.) and increase in weight in successive weekly periods of baby pigs fed on pasteurized or sterilized milk. Second experiment*

Experimental animal			Weight		Weight gain per week					
No.	Sex	Milk	Initial	Final	1st	2nd	3rd	4th	5th	6th
15	♀	Pasteurized	1970	10660	200	110	1190	1780	2360	3050
16	♀	Pasteurized	2090	10930	300	680	1210	1550	2150	2950
20	♂	Pasteurized	1240	10130	360	160	1260	1650	2300	3160
Mean			1767	10573	287	317	1220	1660	2270	3053
12	♀	Sterilized	2020	10250	80	720	990	1670	2140	2630
17	♀	Sterilized	1560	9470	270	670	1030	1290	2010	2640
13	♂	Sterilized	1930	10760	160	590	1090	1640	2430	2920
Mean			1837	10160	170	660	1037	1533	2193	2730

Table 6 presents individual and average weight gains of the surviving animals in the six successive weekly periods of the experiment.

On the whole the growth figures are less uniform than in the foregoing experiment, which undoubtedly may be ascribed to illness and unequal food intake during the first 3 weeks. Weight gains on pasteurized milk were superior to those on sterilized milk except in the second week. The percentage weight gain on sterilized milk in six subsequent weekly periods was 59, 208, 85, 92, 97 and 89% of that on pasteurized milk. For the entire experimental period this value is 95%.

(c) *Third experiment*

One litter consisting of six male and four female baby pigs remained with the dam until 4 days old and was then placed on experiment. Supplementing the milk with the meal mixture was started at the end of the fourth week. One pig out of each group

developed diarrhoea, the one on pasteurized milk died on the ninth day of the experiment, the other on sterilized milk recovered. Another animal fed sterilized milk died at the sixth day without marked symptoms of illness except loss of appetite some days prior to death.

In the first half of the experimental period a difference in appetite was observed in favour of the pasteurized milk. In the second half all animals drank with equal avidity, and the amount of milk fed was gradually increased up to 1000 ml. per animal per feeding in the sixth week, whereas in the foregoing experiments the maximal amount had been only 500 ml.

Table 7. *Initial and final weight (in g.) and increase in weight in successive weekly periods of baby pigs fed on pasteurized or sterilized milk. Third experiment*

Experimental animal			Weight		Weight gain per week					
No.	Sex	Milk	Initial	Final	1st	2nd	3rd	4th	5th	6th
21	♀	Pasteurized	2130	11170	290	640	850	1150	2170	3940
28	♀	Pasteurized	1980	11070	230	710	1080	1160	1960	3950
27	♂	Pasteurized	2020	11850	280	740	1020	1340	2140	4310
29	♂	Pasteurized	1630	12050	420	810	1120	1540	2220	4310
Mean			1940	11535	305	725	1018	1298	2123	4128
23	♀	Sterilized	2010	11700	250	710	1110	1060	2140	4420
26	♀	Sterilized	1350	10840	310	610	860	1130	2220	4360
24	♂	Sterilized	2160	11810	260	800	1130	990	2070	4400
25	♂	Sterilized	1560	10690	120	660	1040	1240	2180	3890
Mean			1770	11260	235	695	1035	1105	2153	4268

Individual and mean figures for weekly gains are given in Table 7. In this experiment growth differences of some importance occurred in the first and fourth week in favour of pasteurized milk. The average growth increments on sterilized milk in successive weekly periods were 77, 96, 102, 85, 101 and 103% of those on pasteurized milk with an average of 98% calculated over the entire experimental period.

In each of the foregoing experiments with baby pigs growth on sterilized milk was less than on pasteurized milk during the first week. Thereafter only small differences of doubtful significance occurred. Obviously the slower weight gain on sterilized milk in the beginning is the result of greater difficulties of the baby pigs to get accustomed to this type of milk rather than of a decreased nutritive value as a result of the heat treatment. The slightly lower weight gains on sterilized milk after adaptation was not sufficient evidence for decreased nutritive value, since they might be effected by greater adaptation difficulties in the initial phase of the experiments. In order to find out whether or not the somewhat slower growth rate of pigs on sterilized milk after adaptation could be ascribed to impaired nutritive value, an experiment was performed in which all pigs were accustomed in the same way to the milk feeding prior to the experimental period. Only thereafter the growth trial on pasteurized and sterilized milk was started.

(*d*) *Fourth experiment*

A number of baby pigs from three different litters varying in age from 1 to 6 days was fed a commercial artificial milk mixture for baby pigs (Milkivit). After 10 days the artificial milk was gradually replaced by pasteurized milk in the course of 4 days. Then

the animals were weighed and divided into two comparable groups according to litter, weight and sex. One group of six animals was fed pasteurized milk, the other group of seven animals was fed sterilized milk for 3 weeks. Small supplements of solid food were added to the milks during the fourth week of the experiment.

After starting the milk feeding no mortality occurred and only one animal in each group developed scouring. In general the sterilized milk was more rapidly consumed than the pasteurized milk.

Individual and average gains in the four experimental periods are presented in Table 8. It is shown by these figures that both milks supported nearly equal growth. Weight gains on sterilized milk in successive weekly periods are 96, 99, 98 and 98% of those on

Table 8. *Initial and final weight and weight gain (in g.) in successive weekly periods, of baby pigs fed on pasteurized or sterilized milk after a uniform preparatory period. Fourth experiment*

Experimental animal			Weight		Weight gain per week			
No.	Sex	Milk	Initial	Final	1st	2nd	3rd	4th
38	♀	Pasteurized	2160	9700	1110	1580	1970	2880
40	♀	Pasteurized	3330	11900	1280	1840	2280	3170
46	♀	Pasteurized	1520	10100	1240	1610	2470	3260
53	♀	Pasteurized	2350	9320	820	1530	2010	2610
37	♂	Pasteurized	2280	10470	1250	1870	2270	2800
45	♂	Pasteurized	1890	11720	1400	1930	2560	3940
Mean			2255	10535	1183	1727	2260	3110
39	♀	Sterilized	2330	10960	1220	1900	2410	3100
44	♀	Sterilized	2520	10430	1180	1470	2280	2980
50	♀	Sterilized	1900	10520	1300	1800	2320	3200
51	♀	Sterilized	1770	10210	1180	1850	2250	3160
54	♀	Sterilized	2250	9850	970	1580	2020	3030
56	♀	Sterilized	2740	10370	960	1720	2080	2870
52	♂	Sterilized	2130	9900	1110	1610	2070	2980
Mean			2234	10320	1131	1704	2204	3046

pasteurized milk, with an average of 98% for the entire duration of the experiment. Contrary to the results of the foregoing experiments with baby pigs no considerable difference between both groups occurred during the first week of the experiment. Obviously this discrepancy is the result of the method of adaptation applied in the present experiment and lends support to the view that the important growth differences during the first week in other experiments were determined primarily by troubles in adaptation and not by a decreased nutritive value of the sterilized milk.

DISCUSSION

The results of the experiments reported demonstrate only small and statistically non-significant differences between experimental groups fed either pasteurized or sterilized milk, except the rat-repletion method which yielded a significant difference in the first period.

The absence of differences in growth of rats fed the three types of milk may possibly be ascribed to the subnormal growth rate. In this respect the literature reveals striking contradictions. Some workers have claimed that in their experiments the weight gain of rats on an exclusive milk diet was equal to that on a complete stock ration (e.g.

Kemmerer, Elvehjem, Hart & Fargo (13); Bixby *et al.* (2)); others, on the contrary, obtained subnormal growth rate (e.g. Roine, Gyllenberg, Rossander & Vuokila (17); Henry, private communication), as was the case in the present experiment. It seems not unlikely that strain differences between the rats used account for the above conflicting results.

Contrary to the results obtained with growing rats, the baby pig gained very fast on a diet of cow's milk only. The increase in weight during 5 weeks amounted to approximately 9 kg., which may be considered as normal for sucking pigs under practical conditions (Weybrew, Stewart, Matzone & Peterson (19); Dyrendahl, Swahn, Björck & Hellving (3); Forshaw, Maddock, Homeyer & Catron (4)). Such a high growth rate was rather surprising, since milk feeding was restricted and governed by the consumption of the baby pig with the smaller appetite.

No apparent differences were noticeable between baby pigs of both milk groups with respect to mortality, incidence of scouring and general health. However, the impression was formed that during the first week the animals on pasteurized milk were somewhat less subject to digestive disturbances than those on sterilized milk. It was suggested that difficulties in adaptation to the latter milk may have contributed to this phenomenon.

In each of four experiments carried out independently the weight gain of baby pigs on sterilized milk was somewhat less than on pasteurized milk, which is not in conformity with the results obtained with rats. These conflicting findings might be influenced by the observed difference in growth rate.

On summarizing the growth data obtained in four experiments with baby pigs, it appears that the weight increments on sterilized milk calculated over the entire experimental period amounted to 97, 95, 98 and 98% respectively of that on pasteurized milk, resulting in a mean value of 97% for all experiments with baby pigs. In addition to the difficulties in adaptation to the sterilized milk observed in the first week, the difference in weight gain of 3% has been influenced by a systematic mistake in equalizing food intake. Determinations of total solids and nitrogen content revealed nearly always somewhat lower values in sterilized than in pasteurized milk. In fifteen samples mean solid contents of 11·47 and 11·57% were obtained, while six samples yielded mean protein contents of 3·53 and 3·64% for sterilized and pasteurized milk respectively. These differences may be ascribed to milk proteins sticking to the wall of the bottle during in-bottle sterilization. Since equalizing food intake was based on milk volume the animals fed sterilized milk received about 1% less dry material or about 3% less protein than did those fed pasteurized milk. Therefore the observed difference in growth of 3% may possibly be ascribed in large part, if not completely, to the lower consumption of milk solids by animals fed sterilized milk.

The several experiments described above failed to demonstrate a serious loss in nutritive value of the sterilized milk under consideration. The biological values obtained, the responses of depleted rats and the weight gains of rapidly growing baby pigs in carefully controlled experiments point to some detrimental effect of the heat treatment applied, though statistical evidence was not obtained. As judged from the amount of the differences found between groups fed either pasteurized or sterilized milk the degree of impairment, if any, may only be of minor importance. On the other hand, it is to be remembered that in the experiments recorded above the different types of milk have been compared only with respect to nutritive value. From the practical point of view keeping quality and bacteriological properties should likewise be taken into consideration. As in

these respects the comparison undoubtedly turns out to the advantage of the sterilized milk, it may be concluded that on the whole properly processed sterilized milk is at least as valuable as pasteurized milk.

SUMMARY

1. The nutritive value of milk sterilized according to the Stork procedure has been studied in laboratory experiments by the method of Mitchell, the rat-repletion method and growth trials with rats and baby pigs.

2. Determinations of biological value of the milk proteins with three groups of six rats by the Mitchell method yielded mean values of 87·0, 88·0 and 85·1 for raw, pasteurized and sterilized milk respectively. The corresponding digestibilities amounted to 96·8, 96·3 and 94·4. The differences were not statistically significant.

3. The relative nutritive value of the three types of milk for the repletion of protein-depleted rats was studied with three groups of eight rats. The average weight gain on feeding daily 40 ml. of either raw, pasteurized or sterilized milk during 5 days was 27·7, 26·3 and 22·9 g. respectively. Again these differences were not statistically significant.

4. No differences in growth rate were obtained if three groups of twelve male and twelve female young rats were fed on raw, pasteurized or sterilized milk supplemented with trace minerals for a period of 6 weeks. The growth rate of rats on exclusive milk diets was, however, far below normal.

5. In growth experiments with baby pigs, fed equal amounts of pasteurized or sterilized milk supplemented with trace minerals, small but statistically non-significant differences in weight gain were found, in favour of the pasteurized milk. The average weight gain on sterilized milk in four separate growth trials amounts to 97, 95, 98 and 98% of that on pasteurized milk. The average 3% less gain on sterilized milk was ascribed partly to more difficult adaptation and less intake of milk solids.

6. These experiments failed to demonstrate a significant decrease in nutritive value of milk sterilized according to the Stork procedure.

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634. THE DETERMINATION OF THE COAGULATING POWER OF COMMERCIAL RENNET EXTRACTS USING AN AUTOMATIC TESTER

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(With 2 Plates)

INTRODUCTION

Rennet strength is conveniently measured by the determination of the time required to coagulate milk under standardized conditions. Experience gained in the routine testing of commercial rennet preparations emphasized that two conditions must be satisfied:

(1) A stable reference standard of rennet *or* a standard milk substrate.

(2) An accurate apparatus for measuring coagulating time.

It was found that raw milk was unsatisfactory as a standard substrate owing to large variations in coagulating time with the same sample of rennet. Nor could these variations be sufficiently eliminated by the use of dried milk preparations to permit such a standard to be adopted.

Attention was therefore directed to the preparation of reference standards of rennet by freeze-drying, using the methods of Greaves (1).

Similarly, an automatic coagulation time recorder was developed modelled on the principle used by Randall (2) in an apparatus for testing heparin. This instrument promised considerable advantages in convenience and accuracy over previously described forms of apparatus (3,4,5,6,7,8,9).

EXPERIMENTAL

Preparation of freeze-dried reference standards

Contrary to the experience of other workers we were unable to prepare a milk substrate suitable for use as a reference standard; a specially prepared rennet was therefore employed for this purpose. Such a master reference standard was prepared by freeze-drying commercial rennet in a special medium after removal of the salt by dialysis. It was stored, after drying to a moisture content of 0·5% or less, at a low temperature (5° C.) in sealed evacuated ampoules.

The medium used was one previously developed for freeze-drying lactic streptococci which were found to survive in it in considerable numbers after several years. It seemed a reasonable assumption that the more robust rennin, dried under similar conditions, would suffer little change on storage. This was found to be true for a number of preparations when stored up to 51 months, the difference between the initial and final rennet concentrations being well within the experimental error of the determination.

Dried rennet was prepared by dialysing the commercial extract through cellophane (P300) against water of pH 5·5 until the salt concentration had fallen to less than 0·5%.

To the de-salted solution was added freeze-dried powdered horse serum, lactose and ascorbic acid in the proportion of 7·0, 7·5 and 0·5 g./100 ml. of liquid; adjustment to pH 5·8 followed and the coagulating power checked. In early preparations convenient quantities of the liquid, depending on its coagulating power, were distributed in 25 ml. ampoules, which were individually immersed in solid carbon dioxide in acetone at −70° C. while being rapidly rotated mechanically. The water was then removed from the frozen mass in the primary drying chamber of a freeze-drier* in 16–24 hr. Secondary drying over P_2O_5 for 48 hr. followed and the ampoules finally sealed and stored at 5° C.

Later work showed that inter-ampoule variation could be much reduced by carrying out the primary freeze-drying in bulk. The resultant powder was rapidly ground and mixed, distributed in 2 g. quantities in tared ampoules, dried over P_2O_5, sealed and re-weighed.

Subsequent experimentation has further shown that modifications of this method can be applied without loss of stability, at least up to 18 months.

These modifications were:

(1) the omission of both ascorbic acid and dried horse serum from the original medium, or

(2) a preliminary purification of the crude extract by the precipitation technique of Hankinson & Palmer (10) using the original medium unchanged, or

(3) a simple freeze-drying of the untreated commercial extract.

Working standards for routine use

In our experience, working standards should be at least 2 years old and stored, when not in use, in nearly full brown glass-stoppered bottles in a refrigerator. If the much more easily and cheaply prepared modification (3) proves to be as stable as the original preparation, as seems probable, it will be possible to increase the frequency of testing of these working standards and finally to dispense with them.

Preparation of the milk substrate

With the development of standardized rennet preparations the need for a standard milk medium would appear unnecessary. It was found, however, that the variations between the coagulating times with different raw-milk samples were too great to permit the use of this substrate. Moreover, the presence of fat produced a soft coagulum not well suited to the test apparatus which was developed for the determination of coagulating time.

Freeze-dried separated milk was satisfactory, but too expensive for routine use, and attention was turned to spray-dried separated milk, reconstituted at 40° C. with distilled water.

Compared with raw milk, heat treatment of the powder during manufacture produces a liquid which is deficient in ionized calcium. This must be added in sufficient quantity to bring the coagulation time within reasonable limits and to harden the resultant gel so as to enable it to operate the paddle mechanism of the tester satisfactorily. Coagulation time and gel strength are known to be proportional to ionized calcium content; for example, it was shown that the logarithm of time is linearly related to the logarithm of ionized calcium content within certain limits.

* W. Edwards and Co. Ltd., Model 3PS.

Addition of soluble calcium salts to the milk during reconstitution upsets the calcium balance of the system. Over a long holding period successive replicates gave steadily decreasing coagulation times which never reached equilibrium and finally produced a precipitate, even when the complication of bacterial action was eliminated with penicillin.

The difficulty can be largely avoided by introducing the calcium with the rennet at the time of testing. The clotting action in the test is rapid, and is completed before the calcium can exert any secondary effect on the salt balance of the system.

Even in the absence of calcium there is still some variability in freshly reconstituted dried milk. It is much less pronounced and acts in the opposite sense, i.e. replicates give increasing times. In one case the increase between the first and last replicates after 2 hr. was 38 sec. This instability is traceable to the heating applied to the milk during spray-drying which alters the ionized calcium balance.

When reconstituted, there is a time lag before the balance is restored, increasing as the pre-heating temperature used in manufacture increases. The powder used should therefore be prepared at the lowest possible temperature, preferably without any preheating (Berridge (11)).

Instability due to previous heat treatment is best avoided by reconstituting the powder without calcium in distilled water at 40° C. and ageing the liquid for 2 hr. at 37° C. before use. The substrate should also be maintained at 37° C. during the whole testing period to avoid errors traceable to raising its temperature from room temperature to 37° C. Under these conditions the substrate is stable for 6 hr. with powders of good bacteriological quality.

Many attempts have been made without success to prepare the substrate so that it would always give the same coagulation time with the same rennet. The variation between batches, though small, was found too great to allow the adoption of milk substrate as a reference standard. Even so, the powder used is preferably stored in 28 lb. quantities so that over a long period unnecessary variation between substrates is avoided.

Development of the automatic tester

Preliminary experiments with an apparatus built to Randall's specifications showed that its reproducibility was too poor for our purpose, the standard error of the mean of 10–17 replicates being of the order of $\pm 5{\cdot}0$ to $\pm 6{\cdot}0$. A standard error of $\pm 1{\cdot}0$ or less was desired and the original design was accordingly modified.

In principle, the enzyme-treated substrate, situated in a vessel (*A*) (Pl. 1) mounted on a turn-table (*B*) rotated by a constant-speed motor (*C*), was caused to flow round an eccentrically disposed paddle (*D*). When the reaction was complete, the gelled substrate, now unable to by-pass the paddle, exerted a pressure on it in the direction of flow. This pressure forced apart the platinum points (E_1, E_2, Pl. 2) between the paddle and the fixed support (*F*), thereby interrupting the current and stopping the motor. The edge of the turn-table was calibrated, so the distance travelled was a measure of the coagulation time.

In the original model, the paddle was hinged between pivots, and the tension necessary to keep the platinum points in contact was maintained by weighting one side of the paddle with an adjustable weight, later replaced by a weak spring. These arrangements proved unsatisfactory, the apparatus being erratically over-sensitive and the hinge exhibiting considerable frictional resistance.

To make the hinge frictionless, both weight and pivots were discarded. Instead, the

paddle was screwed to phosphor bronze strip, thickness 0·003 in. (*G*), clamped between Perspex blocks (*H*), and in electrical contact with the point (*I*) through which passes the secondary circuit (*F*). No part of this suspension was free to move except the rigid paddle at the point where the strip entered the clamping blocks, the freedom of movement depending entirely on the elastic properties of phosphor bronze.

Another drawback in the original model was that mains electricity was used which involved insulation of the interrupter. More serious was the 'arcing' which damaged the points and rendered a sharp endpoint unobtainable.

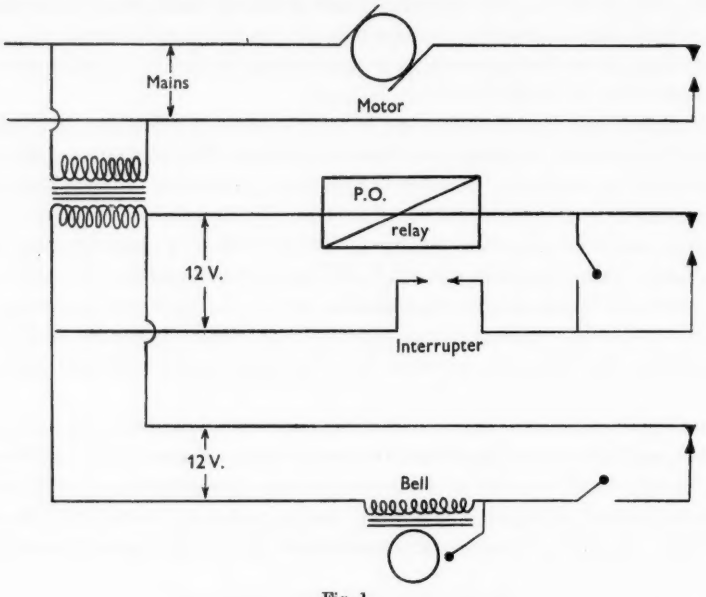


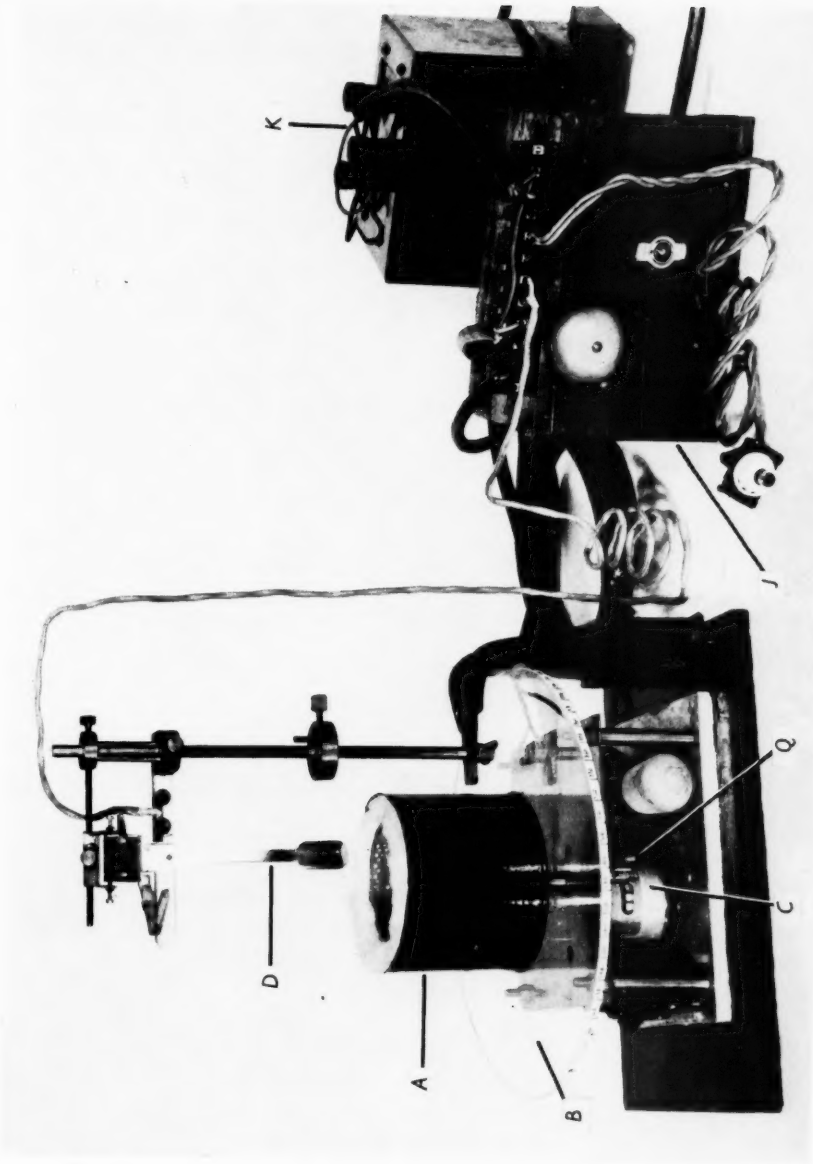
Fig. 1.

For these reasons, the electric current passing through the points was reduced, by a step down transformer (*K*), to 12 V. in a secondary circuit connected through a Post-Office relay (*J*) to the primary mains circuit actuating the motor (Fig. 1). The use of a relay was essential to prevent arcing and chattering after the end-point had been reached. This occurs when the backward pressure of the paddle on the soft gel deforms it and thus allows the platinum points to re-make contact.

Because a low voltage could now be used, the paddle did not need to be insulated, could be made of metal and made part of the secondary circuit. This simplified the design as less insulation was needed, and the use of a paddle, now made of polished steel, greatly increased the accuracy because a smooth surface and rigidity were found essential. Paddles made of Perspex, mica, aluminium and aluminium treated with a heat-hardened protective paint were unsatisfactory.

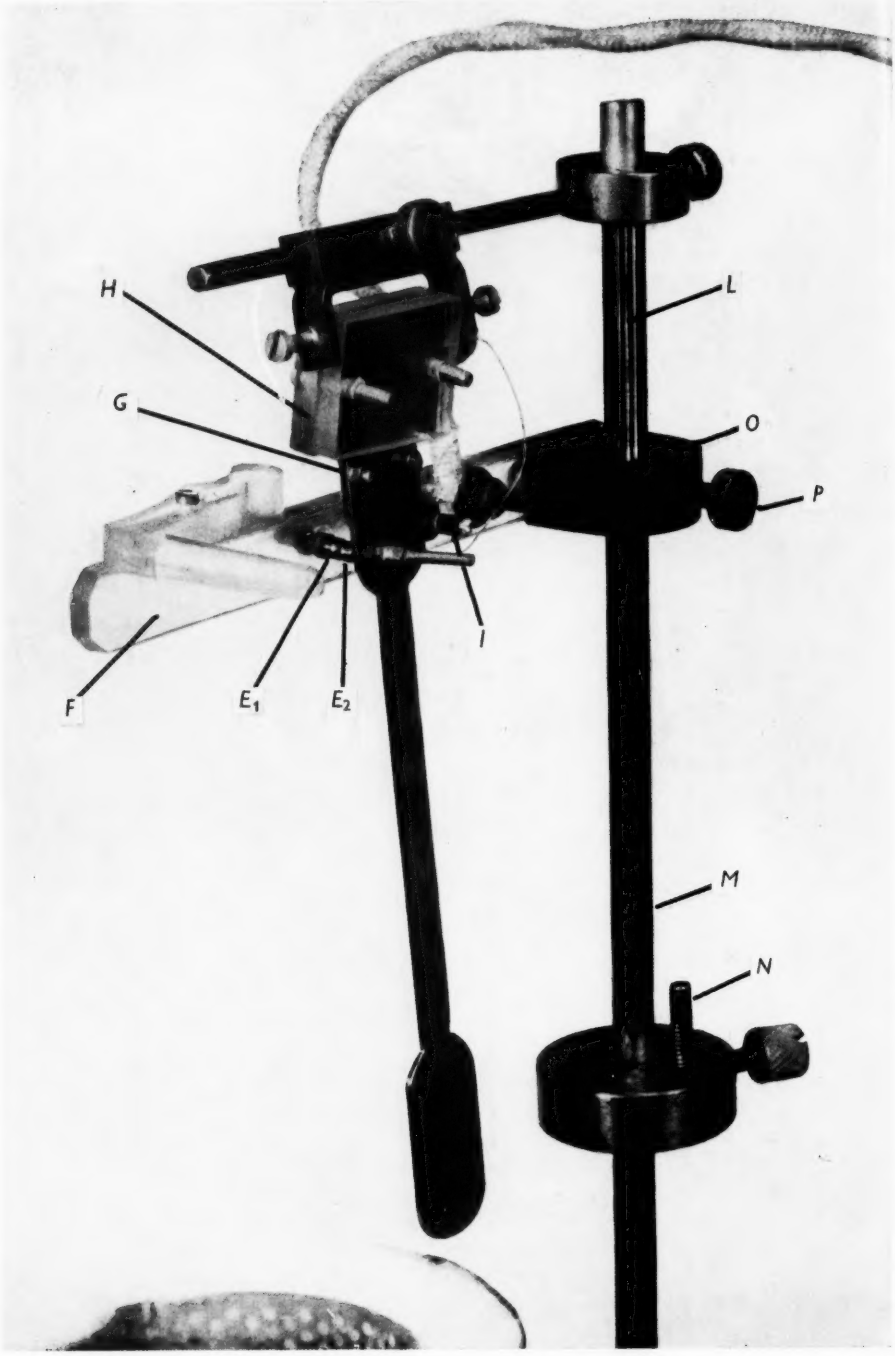
Final form of the modified automatic tester

The model consisted of a circular Perspex turn-table, 22 cm. in diameter, with a periphery graduated into 600 divisions of 1 sec. each moving past a pointer (*Q*). Fixed to it was a circular container with an inner cylinder just wide enough to allow the easy



STORRS—Coagulating power of commercial rennet

(*Facing p. 272*)



STORRS—Coagulating power of commercial rennet

insertion of a 150 ml. straight-sided beaker. The 1 in. space between the inner and outer cylinders was packed tightly with cotton wool to act as a heat insulator.

The paddle (10 × 1·35 cm.) reached almost to the bottom of the beaker and was set at right angles to the rotational flow of the liquid. The top and bottom parts of the suspension were connected together by a rod (*L*) making a rigid unit which could be moved vertically and horizontally about the pillar support (*M*), thus allowing its displacement sideways when mounting the beaker. A metal peg (*N*) in the fixed collar engaged a hole (*O*) in the movable collar, so ensuring that the paddle was always inserted into the beaker to the same depth and at the same angle.

The secondary circuit is maintained through the platinum interrupter, and when broken the bell circuit is completed through the relay, thus giving audible warning that the test is complete. This is necessary as the apparatus must be permanently housed in a 37° C. incubator.

Method of assay

Dilution of concentrated rennet

The standard preparation of freeze-dried rennet is dissolved in 20% saline, delivered from a burette, in the proportion necessary to adjust the resultant solution to an arbitrary rennet concentration of 100%. This figure was set up as the ultimate reference standard to which all subsequent rennets were compared, and it is approximately the strength of ordinary commercial 'standard' extract.

The mixture is allowed to stand 24 hr. in a refrigerator before use and is discarded after 48 hr. Omission of this step may lead to slight errors as dried rennin dissolves rather slowly in saline.

Both this solution and the liquid extract under test are then diluted to 2% with a solution of 5% calcium formate in 20% saline at pH 5·5. Experience has shown that dilute rennet solutions are more stable in concentrated saline, that the pH must be 5·5 to prevent the precipitation of citrates in certain commercial preparations, and that a calcium salt of a weak acid is preferable to that of a strong acid.

Testing

As starting a test from rest introduces an error due to back-lash, the instrument is first switched on. Two millilitres of the 2% rennet solution are pipetted into a spoon, previously warmed to 37° C., and 100 ml. of the warm substrate measured into a 150 ml. beaker. This substrate is raised to 37·5–38·0° C. over a heated wire gauze, removed and stirred rapidly until the temperature is exactly 37° C.

The spoon and contents are at once dropped in and a reading taken from the moving scale of the tester through the closed glass incubator door and the mixture stirred rapidly for 5 sec. The door is opened, the spoon discarded into 10% ammonia and the beaker slipped, without jolting, into the appropriate receptacle after the removal of the beaker already in position. The paddle is then swiftly immersed in the liquid, clamped with screw (*P*) and the doors closed. The whole operation must not exceed 20 sec. duration or the apparatus may be unduly cooled.

When the warning bell rings, a second reading is taken and the paddle at once raised clear of the beaker. Adhering clots are removed from the paddle by immersion in 10% ammonia, assisted by brushing with a small coarse-haired brush, and it is left suspended

and the motor restarted. The beaker containing the spent substrate is left in position until the next test.

Experience has shown that poor reproducibility is nearly always due to lack of control of the temperature, changes of 1° C. in the region of 37° C. producing changes in coagulation time of 10 sec.

Specifically, failure to adjust the milk temperature to exactly 37° C., heating it over 39° C., leaving the incubator doors open too long, using a cold spoon, not cleaning the paddle so that a film accumulates, or jolting the turn-table when inserting the sample have all been found to increase variability between replicates.

Calculation

The strength of a given sample of rennet is determined from the formula

$$\frac{\log t_1 - \log t_2}{a} + \log C_1 = \log C_2,$$

where t_1 is the coagulation time given by a standard rennet of concentration C_1, t_2 is the coagulation time given by a test rennet of concentration C_2 and a is the slope constant.

This formula was based on the findings of Grimmer & Kruger (12), who showed that rennet concentration is an exponential function of coagulation time. Between the limits chosen (0·02–0·06 % in the final substrate) this relationship is linear with a slope constant of $0{\cdot}907 \pm 0{\cdot}017$, determined experimentally on ten samples.

EXPERIMENTAL RESULTS

Instrument error

The instrument error was determined on samples of 10–17 during development in order to demonstrate the variation between replicates of the same rennet dilution with the same substrate. This is a measure of the reproducibility of the machine. It was expressed either as a range or standard deviation, whilst the accuracy of the derived mean coagulation time was expressed as a standard error.

The range (or standard deviation) was found useful for estimating the effect produced by every modification of design or method, and its reduction in size from 60–100 to 5–14 sec. reflected the improvement in machine performance. The standard error, corresponding to the above range given by the final model, was 0·4–0·9. This showed that the mean of 10–17 coagulation times is not likely to be more than 2 sec. in error on a total time in the region of 420 sec.

Sample sizes of the order of 17 are not feasible for routine control purposes, and a sample size of three was substituted as being more convenient. As expected, experiments with three replicates gave reduced ranges of 1–10 sec., leaving the standard deviation substantially unchanged. That ranges of this size can normally be obtained in routine conditions was shown when 258 consecutive commercial samples all gave ranges less than 10 sec. with three replicates, and 65 % gave ranges less than 5 sec.

Five seconds was therefore adopted as the maximum range of triplicates; when exceeded further replicates were taken to give precision to the mean coagulation time. Even with reduced sample size the mean coagulation time was still found to be in error, not more than 3 sec. in about 420 sec.

Total error, with liquid samples of rennet

This error was determined by comparing means obtained from successive samples of three replicates from the same rennet, and represents the combination of errors existing between rennet samples, between substrates and includes the instrument error. It was found not to exceed 2%, and is frequently very much less. This was confirmed in an experiment where a direct comparison was made between known and determined percentages. Eighteen samples of commercial rennet, from three suppliers, were prepared so that their percentage strengths were precisely known. These strengths were then estimated, and all of them gave estimated strengths which were different from the known strengths by not more than 1·2%, and most were much less.

Total error, with freeze-dried rennet in ampoules

Freeze-dried rennet, being a powder, is not as homogeneous as liquid rennet, and testing errors were therefore expected, and found, to be greater. Further, earlier preparations were dried in the ampoule which led to inter-ampoule variation.

If drying has been carried out efficiently, this variation is very small, but it could be detected by analysis of variance. Accordingly, primary drying in ampoules was abandoned in favour of drying in bulk which reduced this variation. Even so, there will still be unavoidable errors associated with the solution of individual ampoule samples.

The errors were determined on samples of two, and then of three, ampoules. Each ampoule was sampled three times and three replicates of each sample tested. There were therefore eighteen samples for a two-ampoule test and twenty-seven for a three-ampoule test. The standard error of the first was found to be about 1·0% or less and of the second 0·5% or less.

In practice, therefore, the estimated mean strength of a freeze-dried powdered rennet in ampoules will differ from its real strength by not more than 2·0% in the first case, or by 1% in the second. These figures are maximum figures and the difference found was usually less. Tests were carried out on samples of six freeze-dried rennets prepared in different ways.

SUMMARY

1. A method for preparing rennet reference standards by freeze-drying is described.

2. A substrate has also been developed using ordinary spray dried separated milk powder which will give reproducible results under specified conditions. These include:

(i) A constant temperature (37° C.) for the duration of the test.

(ii) The exclusion of added calcium ions until actual testing begins.

(iii) The equilibration of the milk at 37° C. for 2 hr. before use.

3. The construction and use of an automatic apparatus for recording rennet coagulation time is also described.

4. The instrument error of a single observation is about 1·0%.

5. With liquid rennet extracts, the total experimental error, based on one sample of three replicates, is 2% or less: with two samples, it is 1% or less.

6. With freeze-dried rennet preparations, the experimental error with two ampoules, sampled thrice with three replicates per sample, was ±2·0%. With three ampoules it was ±1% or less.

The writer wishes to express his thanks to Mr J. E. Adamson and Mr P. Hoare for valuable suggestions during the course of the work, and to Mr J. C. Lord for constructing the instrument.

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635. THE ESTIMATION OF THE TOTAL VOLATILE FATTY ACIDS IN CHEESE

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The estimation of the total volatile fatty acid content of fresh cheese, simple enough in principle, has engaged the attention of dairy scientists for a number of years. One result has been to increase the complexity of suggested techniques, which makes them somewhat unsuitable for dealing with a large number of samples.

Hiscox, Harrison & Wolf(1), in a classical study of extraction methods, finally selected a technique whereby the water washings of the ground cheese, the ether extracts containing the fats from the ground cheese and the ether extract from the protein residue of the cheese were separately examined for their fatty acid contents. The summated figures gave the total V.F.A. content of the sample under examination. Simley, Kosikowsky & Dahlberg(2) criticized this procedure on the grounds that it was lengthy. These latter workers ground cheese samples with phosphoric acid and introduced the material quantitatively into a centrifuge bottle adding thereafter ethyl alcohol, magnesium sulphate (heptahydrate) and ether. After being shaken, the material was centrifuged and the ether layer removed. The procedure of shaking and centrifuging with ether was repeated on two more occasions and the ether extracts combined, extracted with standard NaOH which was then acidified with H_2SO_4 and distilled in the presence of $MgSO_4$ from an 800 ml. Kjeldahl flask. The residual material in the centrifuge bottle was also steam distilled from a similar flask, precautions being taken to stop the distillation when the $MgSO_4$ started to crystallize. The figures for the ether-soluble and water-soluble acids were then combined to give the total V.F.A. Simley *et al.* showed that their method gave results of the same order as those obtained by Hiscox *et al.* and that much time was saved.

The reason for these procedures, involving separations of fat-soluble and water-soluble acids—an arbitrary distinction in any case because water/fat solubility is not a matter of sharp definition—was to ensure that the protein fraction of the cheese was in fact cleared of fatty acid while the retentive power of the fat, on fatty acids dissolved in it, was eliminated (Hiscox & Harrison(3)).

It appeared to the present authors that there was nothing inherently wrong with the simple idea of extracting fresh acidified cheese in a Soxhlet thimble with ether because (*a*) all the V.F.A. are soluble in ether and (*b*), even if they were not, the conditions within the Soxhlet thimble are such that an ether/water partition effect exists. This view of the necessary procedure would be untenable, of course, if the retentive power of cheese protein or amino-acids on fatty acids was, in fact, very great and if losses of V.F.A. during the ether refluxing process occurred as was suggested by Hiscox, Harrison & Wolf(4). More recently, in this latter connexion, Sjöström & Willart(5) have used a Soxhlet extraction process for cheese, but their recovery of V.F.A. has ranged from 85 to 95% with

added acids. With the above considerations in mind the following experiments were conducted, and since the congruence of results obtained by the methods of Simley *et al.* and Hiscox *et al.* was established by the former workers, it was decided to compare their methods with the present one, when comparison became necessary.

METHODS

Cheese. Cheese was obtained in arbitrary fashion with respect to age and storage conditions from the local markets, but, in addition, a number of cheeses obtained from the Dairy Department of the North of Scotland College of Agriculture were made available. This was particularly valuable since some of them were soft cheese varieties which we had found caused difficulty with the procedure of Simley *et al.* when it came to distillation of the protein residue fraction.

Preparation of cheese. About 40 g. of the chilled cheese, free from rind, were passed through a small mincing machine which had itself been kept in a deep-freeze cabinet. Of the minced sample, 10 g. were accurately and quickly weighed into a weighing bottle and transferred to a chilled mortar and pestle, there to be ground with 3·5 ml. of cold 10% H_3PO_4 and an amount of sand (25 g. for soft cheese; 15 g. for hard cheese) which came from a supply kept in a deep-freeze cabinet. The material thus prepared was at about pH 2.

Extraction of cheese. The above preparation was placed in a Soxhlet thimble (27 × 100 mm.), the ether used to wash the mortar being poured over the thimble, once that was in position, and extracted with ether for 8 hr. (overnight). The condenser, at the end of this period, was washed down with ether. The ether was put in a separating funnel, along with the ether washings from the Soxhlet flask, and extracted six times with 25 ml. portions of 0·1 N-NaOH. On acidification and distillation the last NaOH portion was never found to yield any fatty acid. The extraction was performed without vigorous shaking to avoid emulsification and possible hydrolysis of the fat in the ether layer, and the NaOH portions were run into a single 250 ml. graduated cylinder, the total volume of 0·1 N-NaO·H collected being noted. Of this volume, 25 ml. were placed in a beaker and the ether removed by evaporation on a hot-plate. The somewhat concentrated residue in the beaker after cooling was transferred to a 25 ml. standard (wide-necked) flask and the aqueous washings from the beaker added. A few drops of distilled water sufficed to bring the level to the mark on the flask which was then stoppered and shaken. Of this material, 3 ml. was distilled from a Markham micro-Kjeldahl apparatus in the presence of 6 ml. of oxalate buffer (10% (w/v) potassium oxalate + 5% (w/v) oxalic acid). The distillate passed through a filter-paper, contained in a small funnel at the open end of the condenser, into a flask and 80 ml. of distillate were collected. These 80 ml. were titrated with 0·02 N-NaOH using bromo-thymol blue as indicator. This indicator, although not the ideal one, was selected because the colour change is readily observed even in artificial light. The titration was carried forward to the point where the indicator just changed from the intermediate green colour to blue. Two further drops of 0·02 N-NaOH were then added to complete the titration. The Markham apparatus was normal in all respects except that a ground glass cone (B 10), fitted with a stopper (E 10) had been fused into the top of the condenser. Through this were now poured 10 ml. of neutral (bromo-thymol blue) alcohol which passed through the filter-paper into a receiving flask. The alcohol washings, containing

water-insoluble V.F.A., were then titrated with 0·05 N-NaOH using bromo-thymol blue as before. All the titrations were performed with a micro-burette.

The above extraction process represents the final one adopted, and an account is given below of various methods by which it has been tested.

VALIDITY OF EXTRACTION TECHNIQUE

Period of extraction. While an 8 hr. period might seem to be time wasted, it was of course not so, since the runs were started late at night to be ready for the morning. Periods of less than 8 hr. did not give a complete extraction whereas extraction for longer periods had no effect on the results (Table 1).

Table 1. *Effect of extraction time on results for V.F.A. content of samples of the same cheddar cheese*

Extraction time	Total V.F.A. in 10 g. of cheese (as ml. 0·02 N-NaOH)		
	Trial 1	Trial 2	Trial 3
2	10·57	—	—
4	12·83	12·31	—
6	13·00	13·17	—
8	13·35	13·35	13·35
10	13·35	13·35	13·35

Recovery experiments with standard acid mixtures. Two standard volatile fatty acids solutions were made. The first contained acetic acid 0·3506 g., propionic 0·2743 g., butyric 0·2280 g., caproic 0·2927 g. and caprylic acid 0·2689 g., in 50 ml. ether (solution A). In the second solution the same acids were present but not in accurately weighed amounts, and they were dissolved in 50 ml. of neutral alcohol (solution B). The calculated volume of 0·02 N-NaOH to neutralize 1 ml. of solution A was 16·53 ml., while the actual titration figure for 1 ml. of solution B was obtained by taking 1 ml. of the solution in a 50 ml. standard flask and making up the volume with 0·1 N-NaOH. Of this latter solution 3 ml. were then distilled from oxalate buffer in a Markham apparatus. The titration value per ml. of original solution B was found to be 15·00 ml. 0·02 N-NaOH.

A large number of recovery experiments were performed with different cheeses mixed with added standard acid solutions A and B, but the following examples will show the type of results obtained (Table 2).

Table 2. *Recovery of added V.F.A. from extraction of cheese in a Soxhlet thimble*

	Vol. of 0·02 N-NaOH (ml.)
Example I	
Total V.F.A. in 10 g. cheddar cheese by Soxhlet extraction method	= 18·60
Total V.F.A. in 10 g. cheddar cheese containing 1 ml. standard solution A	= 35·65
Standard acid taken	= 17·05
Calculated titration value for standard acid in 1 ml.	= 16·53
Recovery	= 103 %
Example II	
Total V.F.A. in 10 g. Cheshire cheese by Soxhlet extraction method	= 19·63
Total V.F.A. in 10 g. Cheshire cheese containing 1 ml. standard solution B	= 34·39
Standard acid taken	= 14·76
Titration value of 1 ml. of solution B determined as above	= 15·00
Recovery	= 98 %

It is clear from the foregoing that no losses of volatile fatty acid occur in the extraction process where an efficient cooling system is employed.

V.F.A. in the Soxhlet thimble residue. The ether-extracted residue in the thimble was transferred to an 800 ml. Kjeldahl flask and 250 ml. of water added. Magnesium sulphate (35 g.) and 50% phosphoric acid (50 ml.) were added to the mixture which was then distilled to crystallization point. The experiment was repeated, and in each case 280 ml. of distillate were collected. The titre in each case was 0·2 ml. 0·02 N-NaOH after subtracting the figure for a blank run on sand, $MgSO_4$ and H_3PO_4. It is clear that no significant amount of V.F.A. remained in the Soxhlet thimble residue.

CO_2 *in V.F.A. distillate.* The other workers already quoted (1,2) stress the necessity for removal of CO_2 from the distillates involved in aeration and refluxing methods. This is clearly necessary in the operation of their techniques, but it is not necessary with the Soxhlet extraction method if that is followed as described above. The Markham distillation was performed—as is customary—with CO_2-free distilled water; the extracting solution of NaOH was made up from fresh NaOH (Analar) in CO_2-free distilled water. A CO_2 determination on a sample of this NaOH solution which was shaken with acid-free ether, heated on a hot plate and distilled from the Markham apparatus, gave a distillate of very small titration value. In fact, the value was identical with that observed for a boiled distillate obtained in corresponding fashion. Finally, in this connexion, bromothymol blue is not particularly sensitive to CO_2, while aeration of a distillate containing, perhaps, formic and acetic acids may result in a partial loss of these components.

Table 3. *Comparative V.F.A. results obtained by extraction of cheese by the method of Simley* et al. *and by the Soxhlet extraction method*

	Method of Simley *et al.*				Soxhlet extraction technique			
Cheese	V.F.A. in protein residue (ml. N/10-NaOH)	V.F.A. (water-soluble) (ml. N/10-NaOH)	V.F.A. (alcohol-soluble) (ml. N/10-NaOH)	Total V.F.A. in 100 g. (ml. N/10-NaOH)	V.F.A. (water-soluble) (ml. N/50-NaOH)	V.F.A. (alcohol soluble) (ml. N/50-NaOH)	Total V.F.A. in 10 g. (ml. N/50-NaOH)	Total V.F.A. in 100 g. (ml. N/10-NaOH)
Cheddar	0·40	3·00	0·55	39·50	0·25	0·13	$0{\cdot}38 \times \frac{155}{3} = 19{\cdot}63$	39·26
Cheshire I	0·40	1·70	0·35	24·50	0·16	0·08	$0{\cdot}24 \times \frac{156}{3} = 12{\cdot}48$	24·96
Cheshire II	0·55	3·20	0·30	40·50	0·27	0·10	$0{\cdot}37 \times \frac{157}{3} = 19{\cdot}36$	38·72
Coulommier	0·55	1·15	0·15	18·50	0·13	0·08	$0{\cdot}21 \times \frac{154}{3} = 10{\cdot}78$	21·56
Gervais	0·15	1·45	0·35	19·50	0·13	0·06	$0{\cdot}19 \times \frac{156}{3} = 9{\cdot}88$	19·76
Pont l'évêque	0·55	0·90	0·25	17·00	0·09	0·08	$0{\cdot}17 \times \frac{154}{3} = 8{\cdot}73$	17·46
Average total V.F.A.	—	—	—	26·58	—	—	—	26·95

Comparison of Soxhlet extraction with the method of Simley et al. For this purpose six different cheeses were used and two runs per sample were made. One set of analyses was performed by the present method and the other by the technique described by Simley *et al.* The results are shown in Table 3.

The factor used in the penultimate column of that table is based on the total volume of NaOH recovered from extraction of the ether solution of fatty acids divided by the volume used in the Markham apparatus. A point which emerged at this stage was that the handling of the protein residue distillation (Simley *et al.*) was extremely difficult with soft cheeses. It is clear that the results obtained by the Soxhlet extraction method are very similar to those obtained by the method of Simley *et al.*

Combination of Soxhlet extraction with the method of Simley et al. Where the V.F.A. distillate is required for the determination of individual fatty acids, several Markham distillates would need to be collected and concentrated together. To avoid this the Soxhlet extraction and washing of the ether with NaOH are pursued as already described, but the total NaOH extract containing the sodium salts of the acids is placed in an 800 ml. Kjeldahl flask and acidified with 50% H_2SO_4. Magnesium sulphate (35 g.) and 100 ml. water are added and direct distillation is carried on till the $MgSO_4$ begins to crystallize. Comparative results for three cheese types are shown in Table 4.

Table 4. *Comparison of V.F.A. results obtained by extraction of cheese by the method of Simley* et al. *and by a modified Soxhlet extraction technique*

	Method of Simley *et al.*				Soxhlet extraction method (modified)		
Cheese	V.F.A. in protein residue (ml. N/10-NaOH)	V.F.A. (water-soluble) (ml. N/10-NaOH)	V.F.A. (alcohol-soluble) (ml. N/10-NaOH)	V.F.A. in 100 g. cheese (ml. N/10-NaOH)	V.F.A. (water-soluble) (ml. N/10-NaOH)	V.F.A. (alcohol soluble) (ml. N/10-NaOH)	V.F.A. in 100 g. cheese (ml. N/10-NaOH)
Cheddar	0·50	3·15	0·50	41·50	3·25	0·50	37·50
Edam	0·30	1·35	0·40	20·50	1·60	0·35	19·50
Gouda	0·30	1·60	0·35	22·50	2·05	0·30	23·50
Average	—	—	—	28·17	—	—	26·83

DISCUSSION

The foregoing results show that an ether extraction of cheese conducted as described is capable of giving results comparable with those obtained by more complicated techniques and is therefore readily applicable to the examination of large numbers of samples. It is clear, of course, that the ether extraction itself takes 8 hr., but this requires no attention. The actual operation of determining the total V.F.A. in the ether extract takes about 2 hr. compared with our time of 4 hr. for the method of Simley *et al.* It may be mentioned that this latter time is increased if soft cheeses are being examined because, as already stated, the distillation of the protein residue fraction has to be made with special care.

No evidence has been adduced by us to show that in the Soxhlet process any loss of volatile fatty acids occurs over the recommended, or indeed longer, extraction period, while the admitted retentive power of cheese protein for fat has been shown to be irrelevant where the extraction period is long enough to be efficient.

SUMMARY

An extraction method is described for determining the volatile fatty acids in cheese. The method, dependent upon Soxhlet extraction and micro-distillation, is speedy with regard to actual manual operation and gives results in accordance with those obtained by lengthier established methods.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION D. NUTRITIVE VALUE OF MILK AND MILK PRODUCTS

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PART I

INTRODUCTION

For some considerable time, at short intervals, Kon and Henry(1) have faced the growing task of reviewing work on the nutritive value of milk and milk products. While they have never claimed that they covered the literature completely, it is certain that few, if any, relevant references were missed. But the increasing volume of literature, together with a change in *Journal* policy towards subdivision of the subject and shorter reviews has necessitated a different approach to the 1954–5 period covered in the present article. To keep within the limits of space available, the author has had to be much more selective than the writers of the earlier reviews in this series, and to some extent a concentration on specific topics has been necessary. Unbiased selection is difficult and some valuable contributions have doubtless been omitted. Further, much original literature to which previous reviewers have had access is not available in New Zealand. For these omissions and shortcomings the indulgence of author and reader is sought. The present article covers a selection of papers published since the last review by Kon & Henry(1) was completed.

Nutritive value of milk for man

The post-war upward trend in world milk production continued in 1954(2) when production in nineteen primary milk-producing countries showed an increase of approximately 2% over 1953 figures. The same percentage increase was also shown during 1953(3). More important, however, than overall increase in production is the changing pattern of milk utilization. During the post-war period consumption as fluid milk has become the main outlet for milk. This, coupled with the increased cheese production, has been associated with a substantial displacement of butter

by margarine. The conversion of feed into animal products is an expensive operation, and this trend towards replacement of butter with vegetable oils, suitably fortified, and the increased consumption of fluid milk and cheese would seem to the present author to be a highly desirable one. There are indications, however, that the consumption of fluid milk, at present price relationships, may have reached its maximum in many countries of the western hemisphere. If therefore quantity is limited, investigations on factors which influence the quality of milk and its products becomes correspondingly more important.

(*a*) *Adult nutrition*

The nutritive value of milk and milk products has been reviewed in the United States in the third edition of the National Dairy Research Laboratory publication, ***Food Value of Dairy Products*** (4), and also in other countries in references (5), (6) and (7). In addition to data on their chemical composition and nutritive value interesting historical information on the utilization of milk and milk products through the ages is given in reference (8).

Asking the question 'How can we get people to use more milk', Spencer (9) outlines the major changes in consumption of milk and milk products in the United States over the past 25 years and discusses some of the factors responsible. As mentioned already in reference (1), in a given community, milk consumption is probably most closely correlated with income level, and the problems of increasing milk consumption are largely economic ones. This is further exemplified in surveys such as that reported in reference (10). But, in attempting to increase consumption, individual preferences, particularly of the housewife whose attitude influences that of the family, are important factors, and in this connexion much useful information on housewives' attitudes to dairy products is contained in a report of a survey made at the National Institute for Research in Dairying (11). A somewhat similar investigation in France is reported in reference (12).

Flavoured milks are providing a welcome addition to the range of dairy products available on the market and are helping to increase consumption of fluid milk, particularly by children. In parts of Australia, for example, sales reach 5–7 % of total liquid milk sales (13), and the introduction of flavoured milk into the free-milk scheme for schools is suggested. The general preference shown by children for this product is also reported by Charley (14). The preparation of flavoured milks is discussed by Provan (15). Preferences for homogenized milk and possible advantages in its use, particularly for the school milk scheme and in institutions, are arousing considerable interest in New Zealand at present, as are also reports of its beneficial use in cases of milk allergy (16). A United States Patent (17) claims to have overcome the problem of oxidized flavour in homogenized milk by the addition of 5 % unhomogenized milk at specified temperatures.

The nutritive value of sterilized milk is reviewed by Richter (18), who advocates its introduction on a large scale in South Africa following a successful trial in Port Elizabeth. The subject of sterilized and pasteurized milks is reviewed and their properties compared in reference (19). That no appreciable loss of nutritive value occurs on homogenization or pasteurization of milk has been further demonstrated by Bixby, Bosch, Elvehjem & Swanson (20), who found that processed milk and raw milk were equally effective in maintaining growth, reproduction and lactation in three generations of rats.

There is an increasing interest in the fortifying of milk with various nutrients. The subject is reviewed by Maynard (21). Fortification with vitamins A and D will be discussed separately. Apparently, however, fortification brings its own problems. For example, in mineral fortified milk, lipase is not inactivated during pasteurizing due to the protective action of ferric citrate (22).

Some data on the nutritive value of milk in Czechoslovakia are given in reference (23).

Important considerations on the loss of nutrients are raised in an article by Deem (24) in which she discusses the handling and storage of fluid milk by the consumer.

A growing interest is apparent in the nutritive value of some indigenous milk products. Data on the value of some of these (dahi, khoa, kheer and channa) are given in reference (25), together with information on milk production and utilization in India and the composition of the different milks that are used for human consumption. The composition and nutritive value of these

products compared with that of the original milk is discussed by Mani, Lily, Balasubramanian & Basu (26). All the processing methods resulted in some loss of nutrients, fat and casein being least affected. In experiments with rats, Lily, Mani, Balasubramanian & Basu (27) found boiled milk and kheer to be equal in growth-promoting activity followed closely by dahi and less closely by khoa. Growth was poor on channa. Fed as a supplement to a poor rice diet, Lily *et al.* (28) also found milk, and various indigenous products from it, equally effective in supporting growth, reproduction and lactation. The digestibility and biological value of the protein in these products has been investigated by Balasubramanian, Lily, Mani & Basu (29).

(*b*) *Milk in infant nutrition*

Since the primary function of milk is the nourishment of the young animal, some brief mention of recent work in this field seems appropriate. Considerable attention is, of course, being devoted to many underdeveloped or unsophisticated communities where need for improved maternal and infant nutrition is well recognized (30).

The particular problems which many of these areas present both with regard to the nutritional quality of the human milk, resulting from deficiencies in the maternal diet, and to the provision of suitable supplements or substitutes are largely beyond the scope of this review. Generally, however, the value of prolonged breast feeding is recognized, as, for example, in India (31), and a recent report suggests that the adverse effect on milk composition of an inferior maternal diet may not be as great as was previously supposed (32). A useful review of infant feeding practices in certain tropical and subtropical countries is given in reference (33).

No less interesting are the results of surveys and clinical trials in a number of more highly developed countries. Here the composition of human milk has received further attention, and a very full report on variations in the major constituents and factors responsible for these variations is presented in a series of papers by Hytten (34). But one nutritional implication of the sophistication of a society is the increasing use of cows' milk in infant feeding (35). The corresponding decrease in the incidence of breast feeding is apparent from a number of surveys (e.g. references (36), (37), (38) and (39)), and considerable interest therefore centres round the most suitable substitute and the nutritive value of these prepared milks relative to human milk.

The making of comparative infant feeding trials is discussed in references (40) and (41). Apart from work done in institutions (where the number of infants involved is usually small) the interpretation of results of these trials is extremely difficult. A satisfactory criterion of nutritive value would also appear to be lacking since weight gain, as used by most workers, has been criticized on the grounds that at least in premature infants variations in the ash content of the milks may effect water retention (42), and in any event weight gain is more closely correlated with the fat content of milk than with other nutrients (43). It is not surprising therefore that surveys and trials comparing the nutritive value of human milk and various cows' milk preparations both for normal and premature infants have produced somewhat conflicting results (40, 41, 42, 44, 45, 46, 48, 49, 50). However, the fact that would seem to emerge from these results is that human milk does not show, over cows' milk suitably modified and intelligently used, the marked superiority assumed by some workers. It is a field in which there is certainly need for further unbiased scientific investigation.

Differences between human milk and cows' milk with particular emphasis on the bifidus factor have been discussed by György (52) and by Buyze (53), and the isolation of an acid with strong bifidus activity is described by Zilliken, Braun & György (54) (see also Part 2).

MILK FAT

Valuable information on the digestion, absorption, transport and storage of fats is contained in the second volume of Deuel's book on the lipids (55).

The nutritive value of milk fat compared with other fats is attracting less attention than in the past. As mentioned in a review of work by Groot (56), apparent differences in nutritive value are

probably largely associated with other factors such as the carbohydrate of the diet. The influence of other dietary constituents on fat utilization in general is again clearly demonstrated in the work of Aaes-Jørgensen & Dam (57), and the effect of lactose on fat utilization is reviewed in reference (58). Milk fat and its importance as a food is discussed in a review by Taufel (59). Based on rat-growth experiments, the presence of growth-inhibiting substances in a number of fats other than milk fat has been demonstrated by Thomasson (60). The effect of these factors is apparent only at higher levels of fat intake. Investigating the nutritive value of buffalo milk fat, Rao (61) found, on fractionating the fat, that the lowest melting fraction produced better growth in rats and highest liver storage of vitamin A. Rao has also investigated the nutritive value of buffalo fat as a supplement to poor South Indian diets (62).

Work on the chemical composition of milk fat has continued and a large number of minor constituent fatty acids have been identified. Shorland and co-workers have been prominent in this field. The earlier work of this team is reviewed in reference (63) and of their more recent work, the isolation of pentadecanoic acid (64), a fatty acid containing an uneven number of carbon atoms, from milk fat and other widely distributed animal fats is of particular interest.

Much useful information on the composition of cows' milk fat and factors which influence it is contained in a comprehensive text by McDowall (65).

Recent work on the synthesis of milk fats in the mammary gland is reviewed by Glascock (67). In considering the nutritive value of milk fat, increasing knowledge of the source of milk fats, either dietary or through synthesis in the gland, is of considerable value. It may assist in explaining variations in important minor constituents, such as vitamin A, which cannot be related directly to changes in these constituents or their precursors in the diet of the animal, the explanation possibly lying in the diluent effect of fat synthesized in the mammary gland. Recent work (68, 69) on the modification of dietary fats in the rumen is also significant in this connexion.

Detection of vegetable and animal fats added as adulterants to milk fat still constitutes a major problem. Since the nutritive value of these fats is scarcely inferior to that of milk fat the attention devoted to their detection can better be justified on ethical than on nutritional grounds. Detection of adulteration has been reviewed by Keeney (70). General methods such as the Reichert-Meissl and Polenske determinations and their limitations are discussed in references (71), (72) and (73). A report on the sterol acetate method is given in reference (74). Promising newer methods are described in references (75) and (76), while knowledge of the most probable adulterants in particular areas has enabled special tests to be developed (e.g. tests of which the one given for dolphin oil in reference (77) is typical).

FAT-SOLUBLE VITAMINS

General information on fat-soluble vitamins is contained in several recent texts; the fourth edition of Harris's book on the vitamins (78) gives a good overall picture of present knowledge; the second volume of Deuel's treatise on the lipids (55) includes much information on the fat-soluble vitamins; and Sebrell & Harris (79), as Editors, cover vitamins D and K in volume II and vitamin E in volume III of the book entitled *The Vitamins*.

(*a*) *Vitamin A and carotene*

Most laboratories, it would seem, have now published their routine methods for the assay of vitamin A and carotene in milk and its products, and there is a marked decrease in the number of methods appearing in the literature. De reports a fluorimetric method (80). In order to obtain greater uniformity, the wider publication of standard and well tested methods (e.g. reference (83)) seems highly desirable. Since vitamin A in milk fat is frequently estimated in the presence of carotenoids the study of the reaction of antimony trichloride with carotene by Rousseau, Eaton, Beall & Lucas (84) is of considerable interest. While reasonably accurate corrections can be applied for the presence of carotene, chromatographic separation of the vitamin and carotenoids, unless these latter are present in small quantities only, still seems most reliable.

Seasonal variations in the vitamin A and carotene content of milk fat have been reported, the results in most cases being substantially in agreement with those previously reported for similar areas. From Sweden results of surveys extending over several seasons are reported (85, 86). In the latter paper (86) district differences are discussed; they are more pronounced in winter than in summer, due apparently to different feeding practices. The effect of seasonal and local variations in the carotene content of pasture on the vitamin A content of Bavarian butter is discussed in reference (87), the vitamin A in butters from two factories varying from 18 to 34 i.u./g. In a survey covering the period 1944–50, levels determined at monthly intervals in Oregon butters were highest in May and lowest in February, vitamin A ranging from 7·6 to 4·9 μg./g. and carotene from 12·8 to 4·2 μg./g. (88). In Russia, variations in vitamin A have been investigated in relation to season and feeding (89). Average herd values were highest in autumn months (175–300 μg./kg. milk) and lowest in spring (27–50 μg./kg.). No relation between vitamin A level in milk and stage of lactation could be established, the differences that occurred appearing to be associated with feeding. The six-fold change from maximum to minimum levels is surprising, but the overall figures are much more likely than the improbably high ones recorded earlier and quoted in a previous review (90). Extremely low vitamin A levels have been reported in Japanese butters (91). The average winter value for twenty-one samples was 14 i.u./g. and for twenty summer samples only 23 i.u./g. It is suggested that the low values, compared for example with American ones, are due to low carotene intake and to vitamin losses during butter-making. Another report from Japan records similar vitamin A figures and gives a carotene range of 2–5 μg./g. (92). From India, Patel & Patel (93) report the vitamin A and carotene content of the colostrum of Kankrej cows with results substantially as reported for other colostrums.

Factors influencing the vitamin A and carotene content of milk fat continue to attract considerable attention. In the United States, Jones, Weswig, Bone & Magill (94) have confirmed that the carotene intake of cows directly affects the levels of carotene in the blood and of carotene and vitamin A in the milk but not those of vitamin A in the blood; the levels of liver carotene and vitamin A also reflected the carotene intake but were not related to blood or milk-fat levels. Liver carotene levels in cows fed recommended allowances of carotene were low. This is in line with the findings of Byers, Weswig, Bone & Jones (95), who observed that vitamin A and carotene levels were very low in the liver, blood and milk fat of cows that had received for several years 50 μg. carotene/kg. body weight/day. These workers reported also that levels in the milk fat but not in the liver responded to carotene supplements. Further, cows with initially normal values and fed a low-carotene diet maintained milk carotene and vitamin A at the expense of liver stores.

The importance of liver stores of vitamin A in maintaining the potency of milk fat has been investigated also by other workers. Hjarde, Nielson & Porotnikoff (96) found that the vitamin A content of the milk fat was largely independent of the animals' reserves and that the low winter levels in the northern hemisphere were not due to depletion of liver reserves. The liver reserves appeared to depend more on age than on carotene intake over a short period of time. Chanda, Clapham & Owen (97), considering vitamin A alcohol and ester separately, found that the ester and carotene in the milk fat reflected carotene intake, but that the level of the alcohol varied in the opposite direction, being highest when the carotene intake was low. They suggest that the increase in vitamin A alcohol is due to the transfer of liver reserves. Of interest in this connexion is the suggestion that the mobilization of hepatic vitamin A for milk fat is accelerated by pituitary hormones (98).

Hjarde *et al.* (96) have again drawn attention to the inefficiency of carotene utilization by cows. They found the absorption of carotene to be correspondingly incomplete whether the intake was very high or just sufficient, if completely utilized, to meet body needs. In agreement with earlier workers they also found that the utilization of carotene from oil was not appreciably greater than from plant sources. This is supported by Olsson, Akerberg & Blixt (99), who found that only a small proportion of the carotene administered to cows was secreted as vitamin A or carotene in the milk. Thus large differences in intake were not associated with corresponding differences in milk-fat levels. Further, they found no correlation between the vitamin A content of the

liver and that in the milk, so that the only way to obtain winter milk rich in vitamin A is to increase carotene intake or utilization from winter fodder.

The complexity of the problem of carotene utilization is further evidenced from a report by Hansen, Porter, Johnson, Eaton, Beall & Moore (100) in which the effects on vitamin A and carotenoids in cows' milk following the feeding of different forms of alfalfa are studied. Although the carotene content of the artificially dried and chopped form was the same as that of the artificially dried and pelleted form, the milk fat vitamin A and carotenoid levels that resulted from feeding the pelleted form as the sole source of roughage were considerably lower than when the chopped form was fed, the values obtained with the pelleted form being approximately the same as those from a field-baled form containing only one-seventh of the amount of carotene. Other workers (101) feeding either hay or sugar-beet foliage silage as sole sources of carotene, at levels adjusted to give the same carotene intakes, found that the milk fat from the cows receiving hay contained more carotene and vitamin A, although the differences for the vitamin A were not statistically significant. Differences in carotene utilization have also been reported by Worker & McGillivray (102), who found that animals fed fresh white clover produced milk fat considerably lower in both carotene and vitamin A than identical twin mates fed rye grass, although the total carotene intake was the same.

In investigations on the utilization of carotene the high level of the pigment in the plasma of cattle is of considerable interest as a possible source of vitamin A through conversion at a site other than the intestine. It has been shown by Church, MacVicar, Bieri, Baher & Pope (103) and Kon, McGillivray & Thompson (104) that cattle, as distinct from other species investigated, are unable to convert intravenously injected aqueous dispersions of carotene to vitamin A. This may be linked with the fact that cattle, as distinct from the other species investigated, circulate carotene in their blood, and a reasonable conclusion might be that they do this because there is no mechanism for converting it to vitamin A. On the other hand, the recent claim by Bruggemann & Niesar (105) to have effected the conversion of carotene to vitamin A *in vitro* using mammary tissues is of considerable interest.

The poor utilization of dietary carotene by cows and the problems attendant upon increasing the vitamin A potency of milk fat through the diet of the cow has led to considerable interest in the fortification of milk and milk products with vitamin A. Wodsak (106) has reviewed conditions affecting the stability of added and naturally occurring vitamin A in foods, including milk and a number of its products. Fortifying milk would appear to pose certain storage problems such as the 'hay-like' flavour reported by Weckel & Chicoye (107). To combat vitamin A deficiency in Malayan infants associated with the consumption of sweetened condensed milk, Loftus Hills (108) has suggested the fortification of Australian condensed milk with vitamin A, but some Russian work (109) suggests that the addition of relatively small amounts of vitamin A may result in some detriment to flavour on storage.

Fortifying ghee with vitamin A has been investigated in India (110) with particular reference to loss during storage under conditions simulating those that obtain in practice. Neither the amount of vitamin A originally present nor the level added affected the rate of vitamin A loss which did not appear to be great, considering the conditions of storage, except in porous earthenware vessels when destruction was rapid. The loss of vitamin A from fortified milks during the manufacture of dahi (111) and khoa (112) and the distribution of added vitamin A between serum and fat during various methods of ghee manufacture (111) have been investigated by other Indian workers.

Sterilization has little effect on natural or added vitamin A, and the fortification of sterilized milk with vitamin A has been recommended on a commercial scale (113). Hellstrom & Andersson (114) found no loss of vitamin A from milk during homogenization.

The expense of freeze-drying precludes its use commercially for cows' milk. It has, however, been suggested in Germany for the preservation of raw human milk (116), the only vitamin affected being vitamin A of which 17–38% is lost.

The stability of vitamin A during ghee manufacture has been investigated by Shroff, Sampath,

Anantakrishnan & Sen (117), who conclude that little or no loss of vitamin A or carotene should take place during the normal preparation of ghee. Reports on the stability of natural vitamin A in ghee are contained in references (118) and (119). Deuel & Greenberg (120) report somewhat better retention of vitamin A in margarine than in butter stored under similar conditions.

Irradiation with ultra-violet light to increase vitamin D content has no effect on vitamin A (121).

(*b*) *Vitamin D*

Two further reports on the vitamin D content of milk fat relate to Indian butters. Henry & Kon (122), using a prophylactic test on rats, found values of 0·29, 0·41 and 0·56 i.u./g. fat for three South Indian butters. These values are considerably lower than those previously reported for butters from the same area (see references (1) and (90)). Ranganathan (123) also obtained values of 1·20, 1·18 and 1·50 i.u./g. fat for three butters from South India. It would appear therefore that the effect of tropical sunlight on the level of vitamin D in milk fat is less than was previously supposed. The effect of ultra-violet concentration in sunlight on the vitamin D content of milk fat is clearly demonstrated in the work of Bruggemann & Karg (124), who compared the vitamin D content of the milk of cows from alpine pastures with that of cows from the valley and lower alps and with that of typical dairy milk.

The effect of irradiation on the vitamin D content of milk has been further investigated. Coates, Harrison, Henry & Kon (125) in a detailed investigation using rat and chick assays reported vitamin D levels of the order of 350 i.u./l. for milk irradiated by the Scholl-Scheer-Steinheil method. Comparable figures for numerous irradiated milks have been reported by other workers (e.g. references (127) and (121)). The lower values reported by some workers (e.g. references (128) and 129) would appear to be due to inefficient irradiation (as evidenced by the poor conversion of ergosterol suspension reported in reference (129)). The evaluation of the performance of equipment for irradiating milk is discussed by Sauter (130).

Various methods for fortifying milk by the addition of vitamin D are reviewed by Hofmeyer (131) who considers the use of a water-soluble compound of vitamin D and lactalbumin to be the best method. The use of a similar protein-bound form of vitamin D to supplement the vitamin D intake of cows has been recommended by Wauschkuhn (132). A deficiency of vitamin D in the feed with consequent impairment of calcium and phosphorus uptake may, it is suggested, influence unfavourably the composition of milk for cheese-making. Discussing the difficulties of adding vitamin D to milk, Jonxis (133) considers that they are too great for milk to serve as a vehicle and recommends the addition of vitamin D to other products such as margarine. In the light of experience in the United States and Germany, fortifying milk by the direct addition of vitamin D has, however, been recommended in France (115).

(*c*) *Vitamin E*

A rapid modification of the Emmerie and Engel method for the estimation of tocopherol in butterfat is described in reference (51). Interfering vitamin A and synthetic butter colours are removed by treatment with 60% sulphuric acid, and a correction is made for carotene which is not removed by the acid treatment. Nobile & Moor (47), on the other hand, remove the vitamin A with antimony trichloride followed by chromatography on alumina to isolate the tocopherol.

The tocopherol content of Canadian butters has been reported by Anglin, Mahon & Chapman (66), who found mean values of about 41 and 20 μg./g. fat for summer and winter samples respectively. Beckmann (81) reports levels ranging from a trace to 13 μg./g. (mean 4 μg./g.) in German milk. Levels in human milk and colostrum ranging from 2 to 35 μg./g. with a mean of 13 μg./g. are also reported in reference (81).

Most interest in tocopherol still centres round its role as an anti-oxidant. With milk fat the problem is a complex one, and the main need is for a better understanding of the oxidative processes in milk and its products. While tocopherol definitely functions as an anti-oxidant, there are obviously other factors involved. Thus the feeding of 2 g. tocopherol per day to cows doubled the tocopherol content of the milk fat, but there was no improvement in the keeping

quality of the butter during cold storage (82). Studying the effect of feeding roughages harvested at different stages of maturity and preserved as silage or hay, Krukovsky, Trimberger, Turk, Loosli & Henderson (126) found that the resistance to oxidation in the presence of pro-oxidants was not related only to tocopherol content but that the late harvested silage contained some factor that effectively resisted the development of oxidized flavours in the milk.

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PART II

INTRODUCTION

This part of the review of the nutritive value of milk and milk products is concerned with the proteins, carbohydrate and water-soluble vitamins, and includes some discussion of analytical methods and compositional data concerning these vitamins.

Editorial policy has decreed a radical change from the comprehensive reviews published in past years by Henry and Kon, and has necessitated a different and more selective approach to the subject.

A critical appraisal of the relevant work published during 1954 and 1955—from which the material reviewed has been selected—leaves the impression that this period was one of consolidation, with relatively few real advances in knowledge.

Milk proteins

The manufacture and uses of casein, and the manufacture of dried and condensed whey are discussed in detail in a book by Spellacy(1). Problems associated with the supply of purified casein for nutritional research are reviewed in an article by Copping(2).

The approaching saturation of the consumer demand for liquid milk in many European countries and in the United States makes it increasingly necessary to preserve the nutritive value of processed milk products, which are of considerable importance as sources of high-quality protein in many other countries where preserved milk is often the sole protein source of the infant, and in children and adults it has to compensate for the amino-acid deficiencies of a vegetable protein diet, particularly those of lysine, methionine and tryptophan.

Much of the research on the effect on milk proteins of heat treatment has been concerned with the comparison of the biological value for rats or other animals of milk before and after such treatment. The development of microbiological methods for the measurement of amino-acids makes it possible to supplement determinations of biological value with studies of the amino-acid composition of the protein, and of the biological availability of these amino-acids. Heat treatment may reduce the availability to animals of the amino-acids in a protein either by causing their destruction, or by inactivation due to the formation of a complex from which the acid can be released by chemical hydrolysis but not by proteolytic enzymes. Patton (3) discusses some of the nutritional implications of the spoilage of milk products resulting from excessive heat treatment leading to browning and other changes. The Maillard reaction between the ϵ-amino group of lysine (in casein) and reducing sugars is now a well-established cause of the lowered nutritive value of heat-treated milk products. This is confirmed by the careful study by Mauron, Mottu, Bujard & Egli (4) who investigated the changes in the *in vitro* availability of tryptophan, methionine and lysine resulting from boiling milk, and from the preparation of roller-dried and spray-dried milk powders, evaporated milk, and sweetened condensed milk. The only appreciable losses found were of lysine in evaporated milk (18%) and in roller-dried milk (13–26%). Both chemical and enzyme (papain) hydrolyses were carried out, and it was evident that lysine was partly destroyed and partly inactivated.

In model experiments on the interaction of casein and glucose, sucrose, lactose or maltose, Indian workers (5) found that moist casein autoclaved with a sugar for 2 hr. at 15 lb./sq.in. was digested more slowly by trypsin or pepsin than untreated casein or casein autoclaved with water, though a less severe heat treatment (0·5 hr. at 5 lb./sq.in.) did not affect its digestibility. Extending their study to an indigenous milk product they found that the preparation of khowa from reconstituted skim milk caused some loss of enzymatic digestibility, but its preparation from buffaloes' milk did not.

In a study of protein digestion *in vitro* Mertz, Rennert & Cole (6) found that augmentation of pepsin by the proteolytic organism *Pseudomonas aeruginosa* gave digestibility values more nearly approaching those of animals than did pepsin alone.

The inactivation of lysine in heated milk products was again demonstrated by Debaun & Connors, who, in *in vitro* studies (7), showed that the digestibility of whey powders, as measured by the amount of lysine released by tryptic digestion for 24 hr. at 35°, was linearly related to its biological value for rats. These studies showed that the biological value of roller-dried whey was inferior to that of the spray-dried product, and this finding was confirmed by Riggs, Beaty & Mallon (8), who determined protein efficiency ratios for rats and found spray-dried whey superior to the roller-dried product but inferior to lactalbumin. The discrepancy between the spray- and roller-dried products could be partly corrected by the addition of lysine to the latter. Methanol extraction or heat coagulation increased the nutritive value of both whey powders. The biological value, determined with adult rats, of the spray-dried whey was 90–94 and of the roller-dried 82–84. The authors point out that the values for roller-dried whey are higher than would have been expected from the results of growth tests with young rats and are probably due to the lower lysine requirement of the adult rat.

Hodson (9), continuing his studies of the effect of heat treatment on the nutritive value of milk proteins for rats, found that sterilization of evaporated milk caused an average loss of 12·7% in the protein efficiency for growth (g. of wt. gain/g. protein fed). This loss was reduced by supplements of cysteine or methionine. As he had found no difference between sterilized and unsterilized evaporated milk in a rat repletion test (10), Hodson considers that his results reflect the increased

needs of S-containing amino-acids for hair production by the growing rat, and that they should not be taken as implying that sterilized evaporated milk is an inferior product for the nutrition of man.

The interest in the development of microbiological methods of assay during the last decade has included several attempts to devise an assay for the biological value of proteins to replace or supplement the relatively time-consuming and expensive procedures with animals. An important criterion for any such assay is that it should yield results in accord with animal tests. A method that appears promising is that described by Pilcher & Williams (11) for the measurement of the nutritive value of intact proteins based on the growth of *Tetrahymena pyriformis* W.

The growth response was measured by determining the red triphenylformazan formed by the enzymatic reduction of 2:3:5-triphenyl tetrazolium chloride (assessment of growth by measurement of turbidity was found unsatisfactory since some protein suspensions showed decreased turbidity as the proteins were digested). Using casein as a reference protein, the growth-promoting ability of defatted whole egg, peanut flour meal and wheat gluten meal were determined, and gradings referred to casein were in agreement with protein efficiency ratios determined on rats, mice and dogs. The application of the method to the measurement of the nutritive value of crude proteins will be awaited with interest.

The value of the addition of milk solids to bread is well recognized. A suggestion for improving the nutritive quality of bread still further is to add 3% dried skim milk and 3% casein which is claimed to give a dough that is easy to handle and bake to a palatable loaf containing the equivalent added milk protein of 12% dried skim milk (12).

WATER-SOLUBLE VITAMINS IN MILK

General

It is now nearly thirty years since the first, and eight years since the last, member of the vitamin B complex was isolated. The recent announcement of the structural formula of cyanocobalamin by Hodgkin, Pickworth, Robertson, Trueblood, Prosen & White (13) and Bonnett, Cannon, Johnson, Sutherland, Todd & Lester Smith (14) completes the chemical characterization of the recognized water-soluble vitamins. There are, in addition, several well-characterized factors necessary for the growth of certain micro-organisms; these include the Bifidus factor (see p. 296). Claims that various natural products, including whey (15, 16), contain as yet unidentified growth factors for animals await confirmation and the isolation of the factor. General information on the water-soluble vitamins is contained in detail in the three volumes edited by Sebrell & Harris (17), and, more concisely, in the fourth edition of the book by Harris (18).

Analytical methods and compositional data

The concentrations of ascorbic acid and of the vitamins of the B complex in milk and milk products have been determined repeatedly during the last two decades by biological, physicochemical or microbiological methods and a formidable array of data is now available.

There have been no outstanding developments in assay procedure during the past two years. Most workers now use microbiological methods for the measurement of riboflavin, nicotinic acid, pantothenic acid, biotin, folic acid, cyanocobalamin and vitamin B_6, one of the modifications of the thiochrome method for thiamine, and either the indophenol titration method or the dinitrophenylhydrazone method for ascorbic acid. Gregory (19) has published a valuable summary of the methods used for microbiological assays and includes values (her own and from the literature) for the riboflavin, nicotinic acid, pantothenic acid, folic acid, biotin, vitamin B_6 and cyanocobalamin contents of the colostrum and milk of the cow, sheep, sow, goat and human.

Other recent compilations listing the vitamin contents of milk and milk products include (20, 21, 22). The following paragraphs summarize some recent publications listing some determinations of vitamins in milk and milk products. The values reported for cows' milk fall within the accepted ranges.

The vitamin contents of some Indian dairy products, e.g. dahi, khowa, kheer and channa, are given in (23) and (24). Data for the thiamine and riboflavin contents of samples of milk collected from Warsaw milk dealers in (25); the vitamin contents of the milk from Schleswig-Holstein cows in Germany in (26); the effect of uperizing or autoclaving milk on the content of thiamine (reduced by 15 and 30% respectively), riboflavin (reduced by 5% in both treatments) and ascorbic acid (reduced by 9 and 34% respectively compared with its concentration in pasteurized milk) in (27); the riboflavin and ascorbic acid contents of milk in Czechoslovakia in (28). Values for Scandinavian milks and milk products are given for thiamine in (29), for riboflavin in (30) and for nicotinic acid in (31), and in (32) the thiamine, riboflavin and ascorbic acid contents of human milk are compared with those of cows' milk. The folic acid content of milk and milk products are listed in (33). The vitamin contents of the milk of the blue whale are given in (34) and values for riboflavin, nicotinic acid and cyanocobalamin in ewes' milk in (35). The nicotinamide content of Italian cheeses are given in (36) and values for nicotinic acid in human milk in (37) and (37*a*). The effect of microbial action on the vitamin content of fermented milks are considered in (38), (39), (40) and (41).

A seasonal variation in the ascorbic acid content of milk in Japan is claimed in (42) and an influence of the stage of lactation in (43). Values are given for the ascorbic acid content of milk in Finland (44), in Russia (41), (45) (mares' milk (46)), in Denmark (47), in Spain (48) in Italy (49) (ewes' milk (50)).

Vitamin B_6

Although the vitamin B_6 content of cows' milk is two to three times that of human milk, there are several reports that infants given commercial milk preparations have suffered convulsive seizures due to an insufficient vitamin B_6 intake (51,52,53,54). These reports have stimulated renewed inquiry into the stability of the vitamin B_6 in milk to processing. Hassinen, Durbin & Bernhart (55), using *S. carlsbergensis* to measure the vitamin B_6 content of milk products, found that whereas spray-dried skim milk and sweetened condensed milk retained approximately 80% of the original vitamin, sterilized evaporated milk retained only 33–64%. The vitamin B_6 content of the milk was followed through the various processing steps during its incorporation into a heat-sterilized infant preparation; no loss of the vitamin occurred during compounding, pasteurization, homogenization or cold storage, but significant losses occurred during autoclaving the preparation to ensure a sterile product. Further loss of vitamin B_6 occurred during the 4–5 days following autoclaving, but after about 10 days' storage subsequent loss was negligible during the next 12 months. In further experiments these authors showed that infant preparations could be fortified with pyridoxine, which is not destroyed during sterilization. Fortification with pyridoxal or pyridoxamine is not satisfactory, as these compounds are destroyed by heat treatment.

In a subsequent study, Tomarelli, Spence & Bernhart (56) compared the results from microbiological assays using *S. carlsbergensis* with those from a rat growth procedure. They found that only about one-half of the vitamin B_6 content in heat-sterilized milk products, as measured microbiologically, was available to rats. Thus the overall effect of heat sterilization on the vitamin B_6 of milk results in a product which contains only one-sixth to one-third of the biological activity of the original. These findings explain why it is possible for infants receiving heat-sterilized preparations based on cows' milk to suffer from a dietary insufficiency of vitamin B_6, for whereas human milk contains approximately 0·15 mg./l., autoclaved cows' milk may contain only 0·05 mg. of available vitamin B_6 per litre.

Vitamin B_{12}

The vitamin B_{12} content of the colostrum of the cow and goat and of the milks of the cow, goat, woman, sow, rat and ewe were measured by Gregory (57) using *Lactobacillus leichmannii*, a *Bacterium coli* mutant and *Ochromonas malhamensis*. All three organisms gave similar results showing that the vitamin B_{12} of milk is cyanocobalamin and that none of the naturally occurring

analogues are present. However, ultrafiltrates of milk of all six species contained no cyanocobalamin, which indicates that the vitamin is present in a bound form. The bound vitamin B_{12} in cows', goats', rats' and ewes' milk was available to the test micro-organisms, but that in sows' and women's milk was firmly held and was not released by autoclaving with cyanide though digestion with papain rendered it available.

Gregory & Holdsworth (58,59,60) have continued their study of the nature and properties of the binding substances present in sows' milk and other materials. Present indications are that some degree of species specificity governs the availability of bound cyanocobalamin (61).

Further values are given for the vitamin B_{12} content of cows' milk in (62), (63) and (64), of ewes' milk in (65), and of human milk in (64), (66), (67) and (68).

Bifidus factor

The growth factor for *Lactobacillus bifidus* var. *Penn.* present in human milk and in the milk of other non-ruminants, but practically absent from the milk of ruminants is being further studied by György and his co-workers (cf. (69, 70)).

Fractionation of human milk yielded several fractions having different growth promoting activities for *L. bifidus* var. *Penn.* Approximately one-half of the activity was dialysable and the fractions were composed of glucose, galactose, fucose and *N*-acetyl-glucosamine. The non-dialysable portion with high microbiological activity contained a mucopolysaccharide with acid properties composed of glucose, galactose, fucose, *N*-acetylglucosamine, *N*-acetylchondrosamine and neuraminic acid (71, 72).

Bifidus factor activity is possessed by a number of mucopolysaccharides containing *N*-acetyl-glucosamine combined with other sugars (73) and closely related to the blood-group substances (74). A crystalline disaccharide (75) and crystalline tetrasaccharides (76,77) having bifidus factor activity have been isolated. A series of *n*-alkyl-*N*-acetyl-β-D-glucosamidines prepared by synthesis had high bifidus factor activity, and it is interesting to note that the α-isomers were inactive (78,79).

The significance of the bifidus factor in nutrition is not yet firmly established, but, as György suggests, it seems possible, at any rate on teleological grounds, that the presence of the bifidus factor in human milk may facilitate the establishment of the bifidus flora early in the neonatal period. It should be emphasized, however, that many strains of *L. bifidus* do not require the bifidus factor for growth. Furthermore, the bifidus factor may be inactivated by the action of enzymes present in saliva, in *Clostridium* cultures and, somewhat paradoxically, in cultures of *L. bifidus* var. *Penn.* (69). Nonetheless, it has been thought worth while to patent the addition of bifidus factor, or suitable sources of it, to infant foods (80,81,82).

LACTOSE AND FERMENTED WHEY

The industrial utilization of whey and buttermilk in the food and feeding-stuffs industry are reviewed in (83). The study of methods for removing or decreasing the lactose content of whey continues. Enzymic hydrolysis of lactose by a lactase in yeast converts it to galactose and glucose. Whey treated in this manner was dried and the product used for ice cream, processed cheese or feeding-stuffs (84,85). The method was also used to hydrolyse 25% of the lactose in a concentrated skim milk prior to incorporation in ice cream (86).

Further studies of the dried whey prepared from liquid whey fermented by *Torula cremoris* showed that in feeding trials with mice its protein supplementary value approached that of dried skim milk and equalled it when methionine was added. Yeast whey compared favourably with dried brewers' yeast as a source of all the vitamins of the B complex except thiamine (87).

MILK AND MILK PRODUCTS FOR THE PRE-RUMINANT CALF

Although milk is the natural food of the new-born calf it is known that a diet of milk alone does not allow calves to grow and thrive for more than about 5 months. The reasons for this are still not fully understood (88), but the inadequate magnesium content of milk is an important

factor(89,90,91). Under traditional methods of husbandry calves are fed whole milk for about 10 weeks, and they are also given access to hay and a concentrate mixture which they normally begin to eat during the second or third week.

As whole milk is a valuable commodity there has been considerable interest for some years in developing a satisfactory and less expensive substitute that can replace it in the diet of calves at the earliest possible age, preferably immediately the calves have suckled colostrum from their dams. This work has gained renewed impetus in recent years, since dried skim milk and dried whey have become relatively cheap and readily available for use in animal feeds, and it is, perhaps, opportune to comment briefly on some aspects of the formulation of milk replacement diets for young calves.

Calves are, in general, reared either for meat (veal or beef) or for breeding and subsequent lactation (herd replacement). The dietary requirements of animals destined for early slaughter are the more exacting, since it is desirable to maintain a high rate of growth throughout life. With other calves there is nothing to be gained by rapid growth during the pre-ruminant period; the objective should be a healthy animal starting to ruminate at an early age.

Colostrum has been successfully used to replace whole milk(92,93), but whilst such a procedure is useful in avoiding waste it is obviously of restricted value to most farmers, since colostrum is only available at irregular intervals and in limited quantities.

The simplest substitute for whole milk is liquid skim milk which has been used as a feeding-stuff for many years, particularly in Denmark, but difficulties of transport make it of limited application in other countries. Although the calf has a definite requirement for fat it can be reared successfully on a skim-milk diet provided that roughage is available(94,95). The present-day price of dried skim milk makes it possible in the U.S.A. to feed calves from 3 days old on a diet of reconstituted dried skim milk supplemented with vitamins A and D and with aureomycin, and show a saving of 35% in cost as compared with the feeding of whole milk(96). From a nutritional standpoint such a diet is unnecessarily rich in protein, and most formulas for milk-replacement diets, though based on dried skim milk, include cereals and dried whey as sources of additional energy. This additional energy can also be supplied by adding fat, but the incorporation of fat into milk-replacement diets presents practical problems on the farm, though not on the Experiment Station that possesses a homogenizer. Owen(97) has reviewed the results obtained with a number of milk-replacement diets, further papers include (98), (99), (100), (101), (102) and (103). Calf rearing has undoubtedly been greatly helped by the use of antibiotic supplements, and such an addition is commonly made to the feed of calves in the United States, and the role of antibiotics in ruminant nutrition is excellently reviewed in (104) and (105). Whilst there is no evidence that antibiotics influence the nutritive value of calf diets, it is evident that antibiotics may increase the growth-rate of calves and reduce the incidence and severity of scouring. The mode of this action of antibiotics is not yet understood, and their incorporation in calf diets is not, so far, sanctioned in Britain.

Much of the published work on milk-replacement diets is of an *ad hoc* nature. There are still gaps in our knowledge of the nutritive requirements and digestive capabilities of the young calf. When these gaps are filled it should be possible to develop a milk-replacement diet based largely on feeding-stuffs of vegetable origin. Such a diet would remove uncertainties about supplies of dried skim milk continuing at the present cheap price, and should open up the possibility of rearing calves more cheaply both for slaughter to provide veal or 'baby' beef and as herd replacements.

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